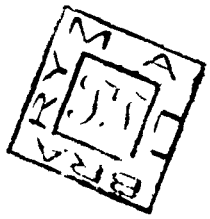
STUDIES ON SEED FATS AND FATTY ACIDS

(RESUME)



THESIS

SUBMITTED FOR THE DEGREE

Doctor of Philosophy

IN

Chemistry

BY

MUKESH BABU

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1981

RESUME

The work described in this thesis consists of two parts.

Part - I deals with the compositional studies on herbaceous seed oils.

Part - II concerns with the reactions of terminal and penultimate acetylenic fatty acids with perbenzoic acid, hypobromous acid, and iodine azide.

PART - I

Uncertainty over the future supply and cost of petrochemicals has rekindled industrial interest in natural seed oils as an alternative raw materials source. In order to overcome the acute shortage of oils and fats, a programme of analysing the herbaceous seed oils is underway in auther's laboratory. Compositional studies of some herbaceous seed oils is given in this thesis.

1. Herbaceous Seed Oils

The oils from the seeds of ten species representing eight botanical families have been examined by various chromatographic and spectroscopic techniques to determine their fatty acid composition.

The gas-liquid chromatographic (GLC) analysis revealed the presence of conventional fatty acids in varying proportions. The amount of total saturated acids present in the seed oils ranges from 13.6 to 28.5%. The palmitic acid forms over 20% of the mixed fatty acids in four species; 24.7% (*Rhynchosia aurea*), 24.1% (*Lathyrus aphaca*), 21.3% (*Ipomoea carnea*), and 20.3% (*Osbeckia chinensis*). The amount of stearic acid, which is usually found as minor constituent of the seed glycerides, is 6.9% in *I. carnea*.

All the seed oils exhibit good sources of C_{18}-unsaturated acids (> 70%). Oleic acid was found in sufficiently high concentration (42.3%) in *Sesamum indicum* seed oil. Oils of *Acanthospermum hispidum* and *O. chinensis* were found to contain a high amount (> 60%) of linoleic acid and hence classified as 'linoleic-rich drying' oils. *L. aphaca* and *Sapium insigne* seed oils contain moderate proportions (~60%) of linoleic-linolenic acids and may be of value as 'drying' oils. Four seed oils (*Sesbania aegyptica*, *Polygonum serrulatum*, *S. indicum*, and *Triumfetta rhomboidea*) containing linoleic acid 40-60% are 'semidrying' oils. Whereas *R. aurea* and *I. carnea* seed oils contain ~ 35% of linoleic acid and may serve as 'non-drying' oils.

The present work revealed that the species rich in oils as well as in specific acids could be further subjected to agronomic evaluation.

2. Cruciferae Seed Oils

Recently much interest is centred in discovering new strains of Crucifers which could yield zero or low erucic acid oils. In the present screening, the GLC analysis disclosed that the oil from Iberis amara is rich in erucic acid (43.4%), a constituent typical of many Cruciferae. On the other hand I. odourata seed oil was found to contain eicosenoic acid (41.9%) but devoid of erucic acid. However, the general composition of conventional acids is usual in both instances. The oil of Cheiranthus cheiri contains both eicosenoic (11.5%) and erucic (22.9%) acids along with eicosadienoic acid (2.3%) as minor constituent. The sufficient oil content (26.0%) and resemblance of fatty acid composition of I. amara seed oil with that of mustard oil warrant further studies to appraise its crop potential and to assess its practical value for providing 'new' edible oilseed. The zero-erucic oil of I. odourata is an interesting finding.

3. HBr-reactive Fatty Acids in Abutilon indicum (Malvaceae) Seed oil

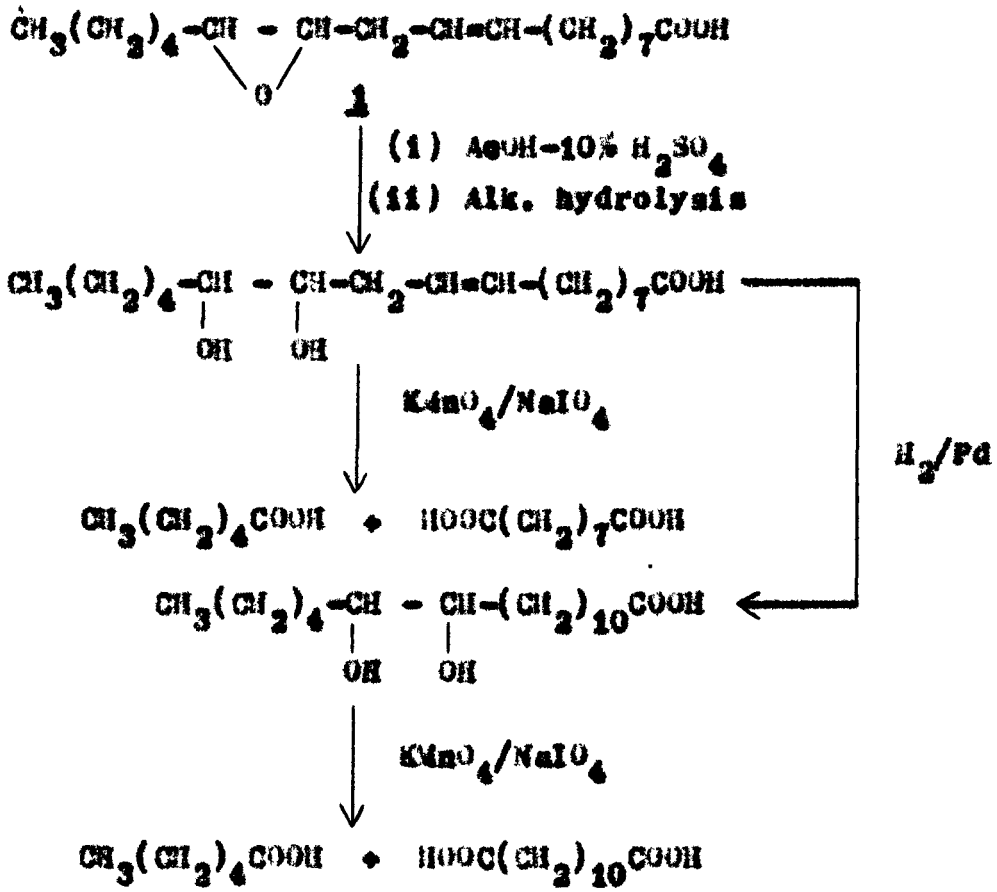
Durbetaki titration of Abutilon indicum seed oil showed it to contain HBr-reactive fatty acids (5%).

The concentrate of non-oxygenated fraction responded to Halphen test for the presence of cyclopropenoid fatty acids (CPFA). The cyclopropenoid acids were characterised as a mixture of

malvalic (2.3%) and sterculic (0.9%) acids along with normal fatty acids by the GLC of the silver nitrate-methanol treated methyl esters.

Successive crystallisations of the crude oxygenated fatty acid concentrate afforded <u>threo</u>-12,13-dihydroxy oleic acid. From this and other evidences it is concluded that 12,13-epoxyoleic (vernolic) acid (1) is present as a constituent (1.6%) of the glycerides. The chemical reactions outlined in Chart I were performed to establish the structure of vernolic acid.

<u>Chart I</u>



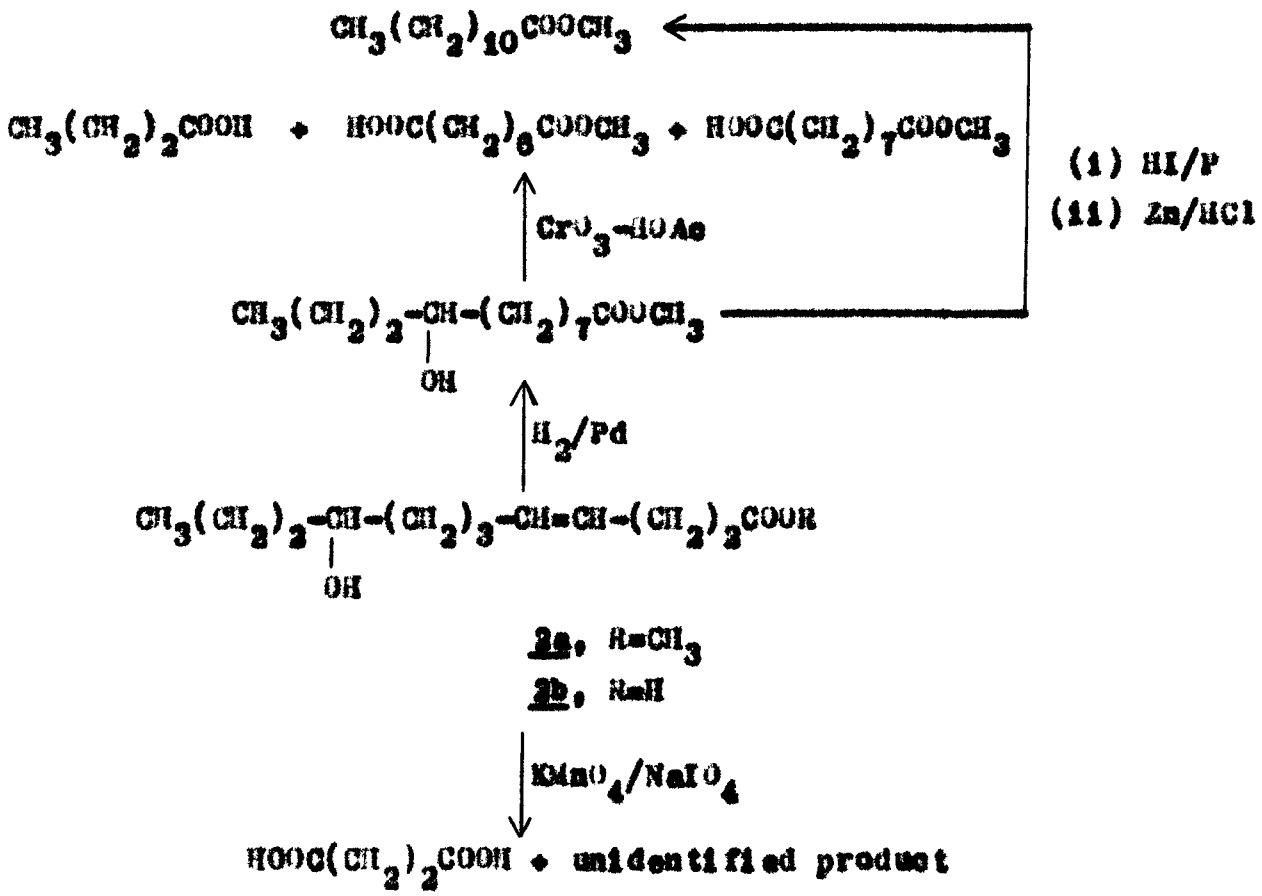
The presence of minor proportions of these biologically

active fatty acids in *A. indicum* seed oil is interesting and deserves further research in lipid biogenesis.

4. A New Short-Chain Hydroxy Fatty Acid in *Anisochilus carnosus* (Labiatae) Seed Oil

Seed oil of *Anisochilus carnosus*, hitherto unexamined species, was found to contain a new short-chain monohydroxy unsaturated fatty acid (1.8%) in addition to the normal fatty acids. The hydroxy ester (**2a**) was isolated by column chromatography and characterised by its spectral behaviour and chemical transformations (Chart II) as methyl 9-hydroxy-*cis*-4-dodecenoate.

Chart II



PART - II

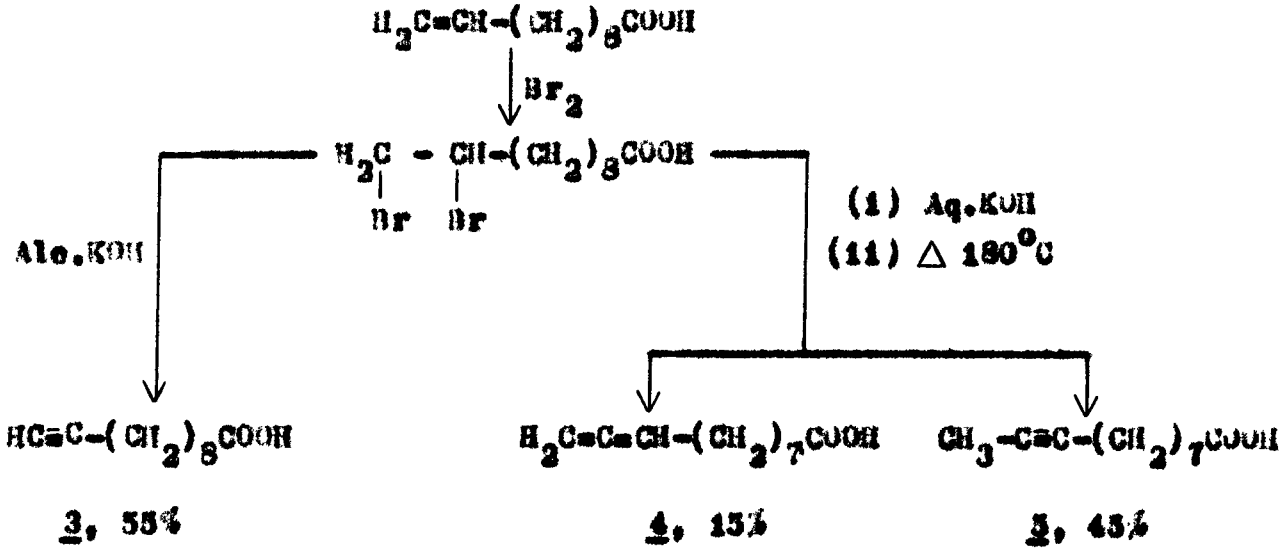
Reactions of Acetylenic Fatty Acids

As acetylenic acids have attracted less attention in the study of fatty acid chemistry, two acids, 10-undecynoic (3) and 9-undecynoic (5), have been prepared by the usual bromination-dehydrobromination method (Chart III).

1. 9,10-Undecadienoic Acid: A Co-Product During Synthesis of 9-undecynoic Acid

A co-product, hitherto unreported, obtained during acetylenic acid (5) synthesis (Chart III), was isolated and characterised as 9,10-undecadienoic acid (4) by combustion and spectral data.

Chart III



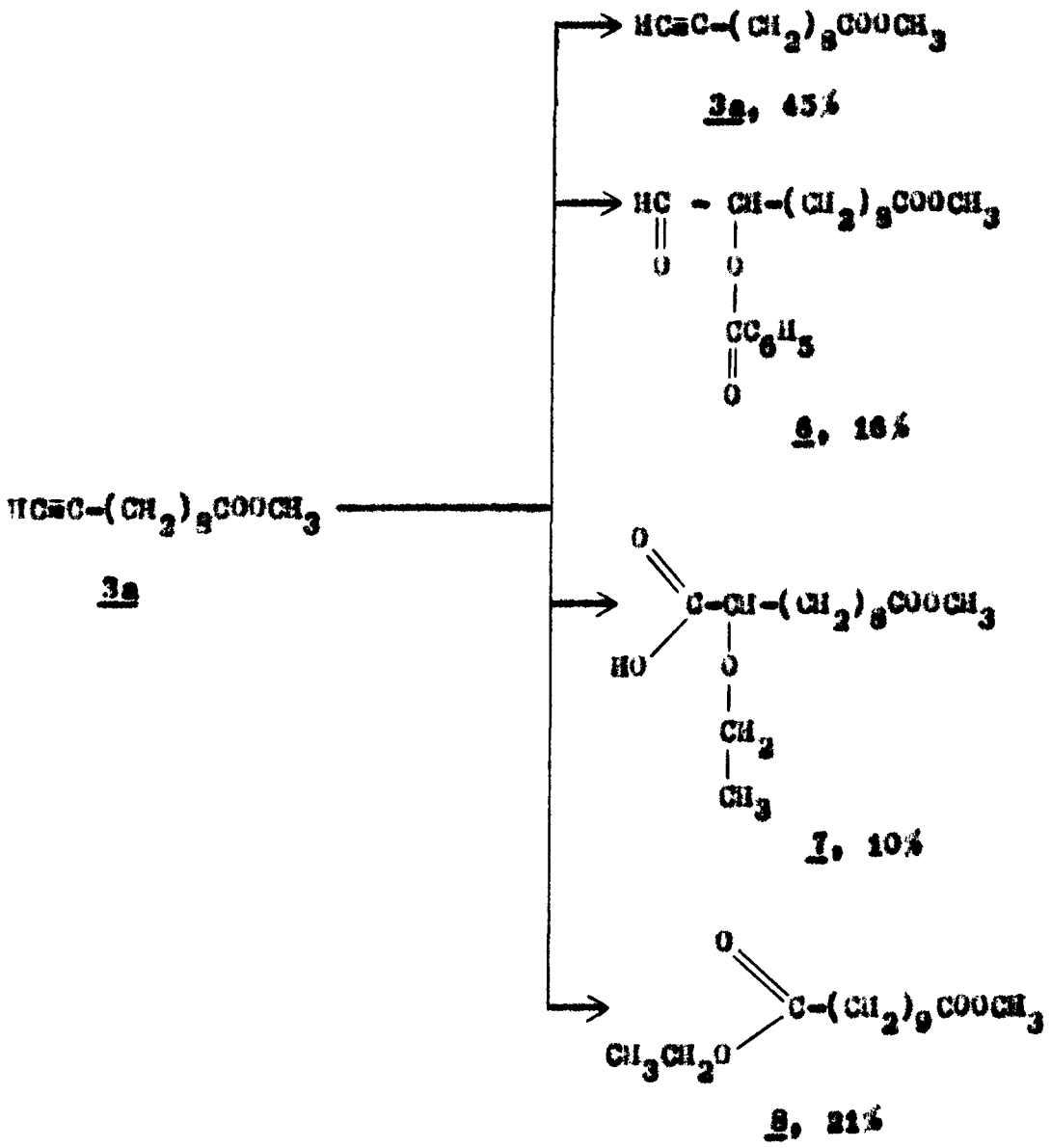
2. Epoxidation Studies of Acetylenic Fatty Acids

With an objective of preparing oxirene (oxacyclopropene) and/or dioxabicyclobutane derivatives, the perbenzoic acid (PBA) oxidations of methyl 9- and 10-undecynoate were investigated. Attempted epoxidation of the acetylenic esters resulted in the formation of rearranged and addition products, instead of yielding the anticipated epoxides. It was observed that the products of PBA oxidations of these esters can be most conveniently accounted for as arising from the oxirene intermediate.

PBA oxidation of methyl 10-undecynoate

The reaction of methyl 10-undecynoate (3a) with perbenzoic acid in chloroform gave unreacted component (3a, ca. 43%), methyl 11-aldehydo-10-benzoyloxyundecanoate (5, ca. 16%), half methyl ester of 10-ethoxyundecane-11,1-dioic acid (7, ca. 10%), and ethyl,methyl undecane-11,1-dioate (8, ca. 21%) following the path depicted in Chart IV.

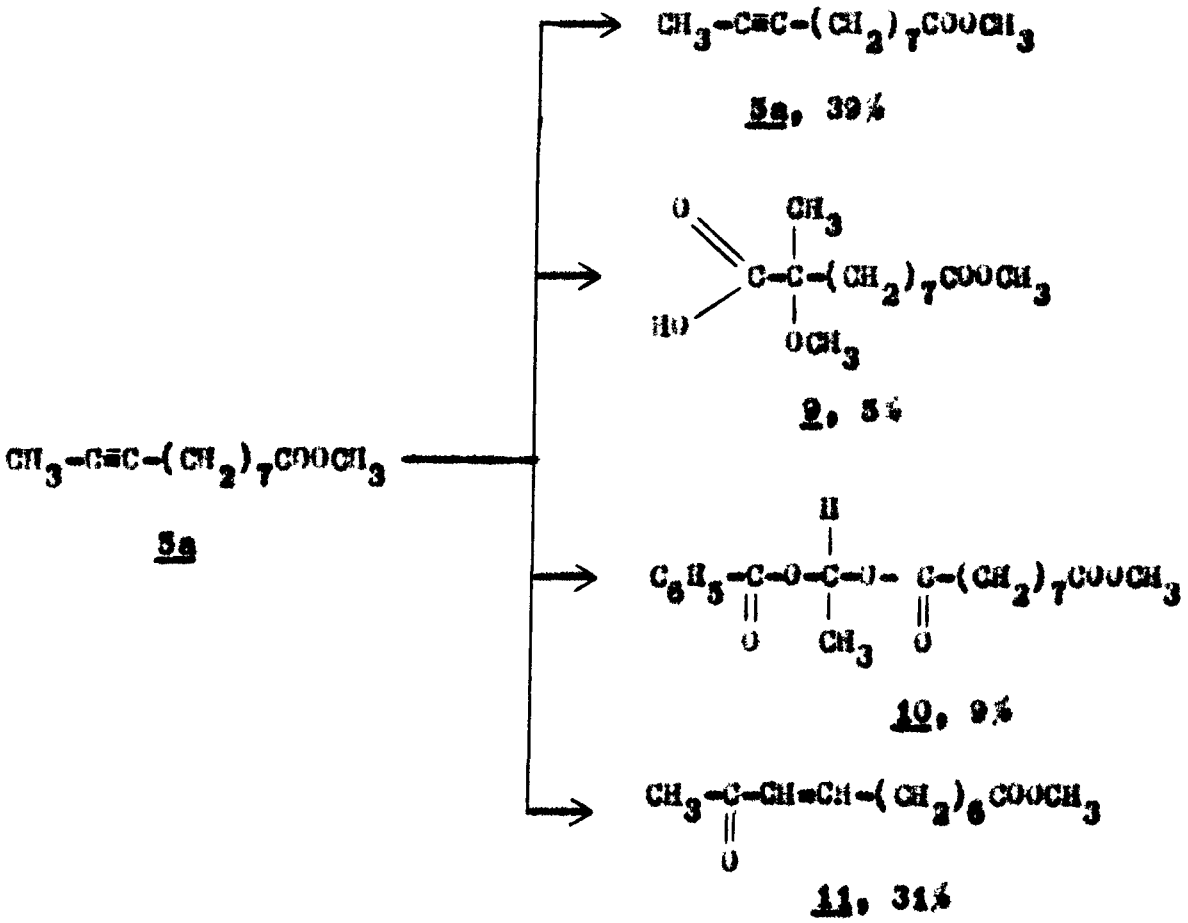
Chart IV


HC≡C-(CH2)8COOCH3
3a
HC≡C-(CH2)8COOCH3
3a, 45%
HC - CH-(CH2)8COOCH3
O O
CC6H5
O
6, 18%
O
C-CH-(CH2)8COOCH3
HO O
CH2
CH3
7, 10%
O
C-(CH2)8COOCH3
CH3CH2O
8, 21%

PBA Oxidation of methyl 9-undecynoate

Similar treatment of methyl 9-undecynoate (5a) with perbenzoic acid gave unreacted ester (5a, ca. 39%), half methyl ester of 9-methoxy-9-methyldecane-10,1-dioic acid (9, ca. 5%), methyl 8-ethylcarboxy(1'-benzoyloxy)octanoate (10, ca. 9%), and methyl 10-oxo-cis-3-undecenoate (11, ca. 31%). The above reaction is outlined in Chart V.

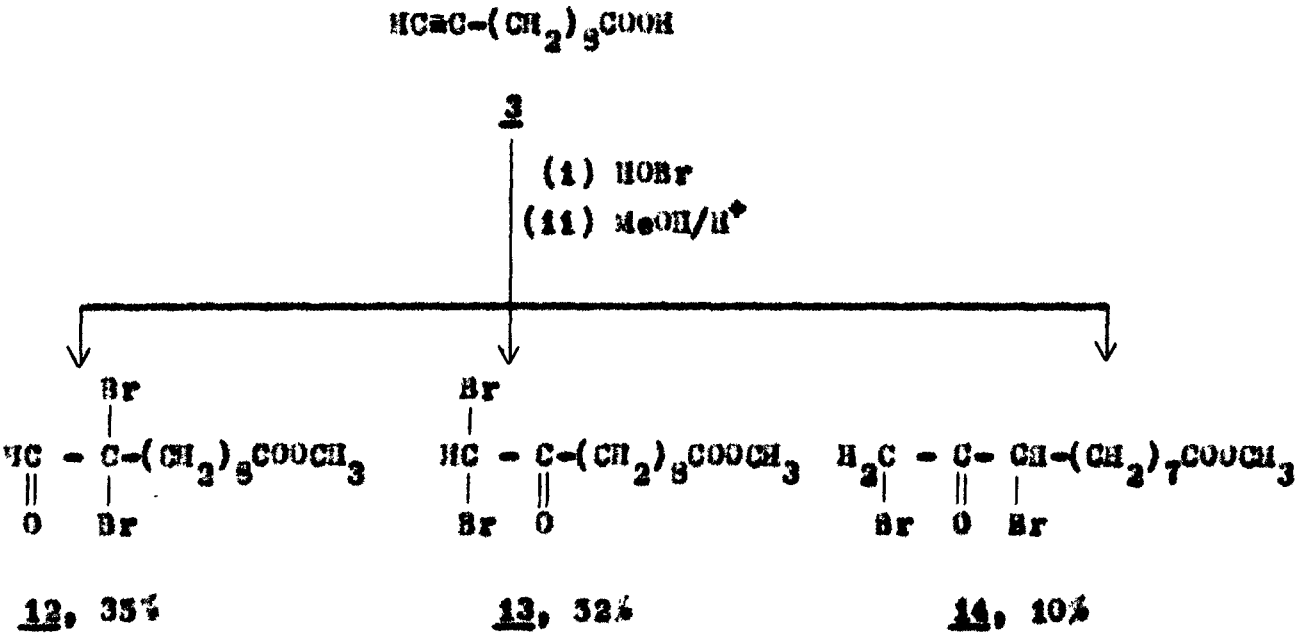
Chart V



3. Hypobromination of Acetylenic Fatty Acids

The reaction carried out in the present investigation (Chart VI) involves the addition of freshly prepared solution of hypobromous acid (HOBr) to 10-undecynoic acid (3). The resulting products after methylation yielded methyl 11-aldehyde-10, 10-dibromoundecanoate (12, ca. 35%) and methyl 11,11-dibromo-10-oxoundecanoate (13, ca. 32%), and one minor component namely, methyl 9,11-dibromo-10-oxoundecanoate (14, ca. 10%). A mechanism is proposed to account for the formation of reaction products in relative yields.

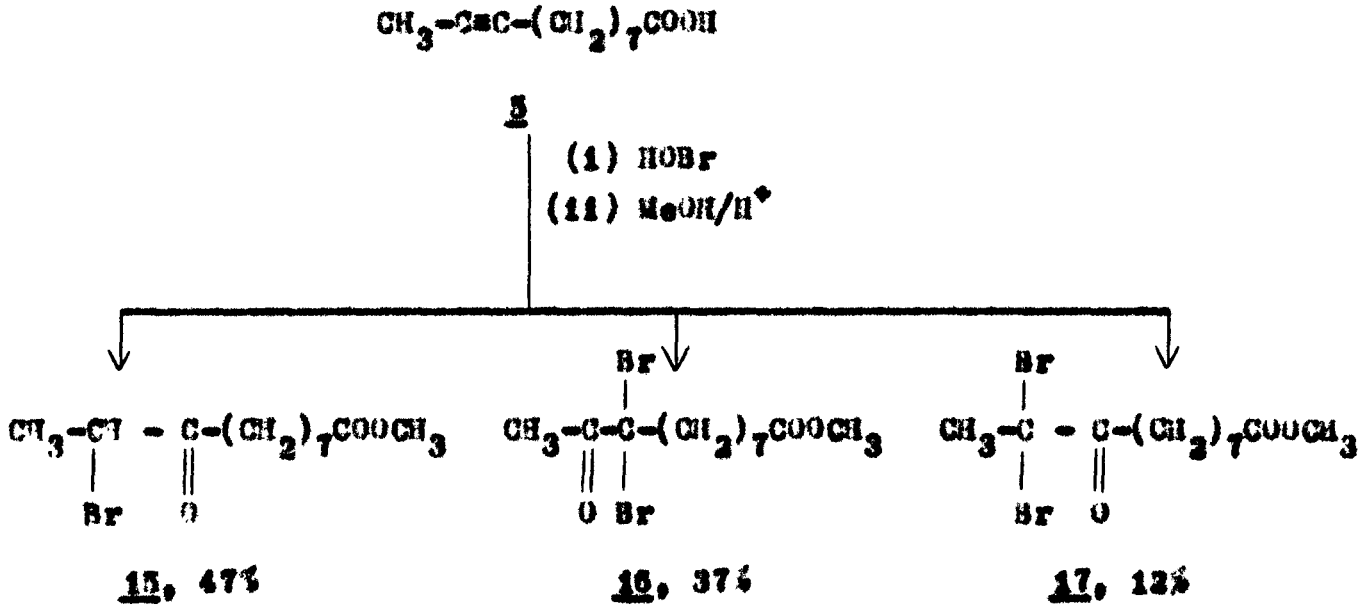
Chart VI



Similarly, the hypobromination of 9-undecynoic acid (5) followed by methylation (Chart VII) gave three products namely,

methyl 10-bromo-9-oxoundecanoate (15, ca. 47%), methyl 9,9-dibromo-10-oxoundecanoate (16, ca. 37%), and methyl 10,10-dibromo-9-oxoundecanoate (17, ca. 12%). To account for the formation of hypobromination products and their relative yields, a mechanism is also proposed.

Chart VII



15, 47% 16, 37% 17, 12%

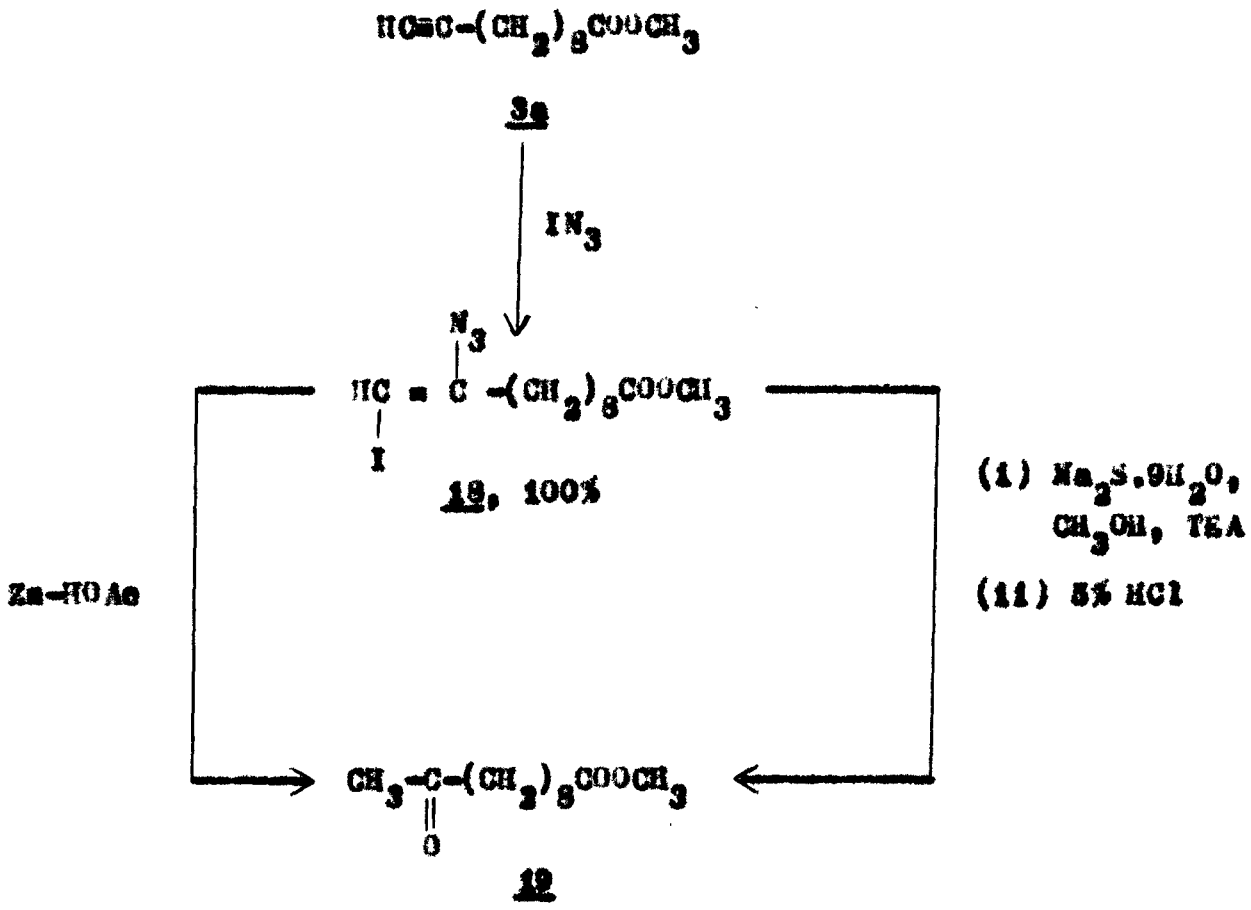
4. Preparation and Reactions of Vinyl β-Iodoazide Derivatives

(a). Of Methyl 10-Undecynoate

The addition of iodine azide (IN_3) to methyl 10-undecynoate (3a) was observed to proceed in a highly regiospecific manner. This addition led exclusively to the formation of 10-azido-11-iodoundecenoate (18) in nearly quantitative yield. The reaction of vinyl iodoazide (18) with zinc in aqueous acetic acid as well

as with sodium sulphide in methanol in presence of catalytic amount of triethylamine (TEA) furnished 10-oxoundecanoic acid (12) in 70 and 90% yield, respectively. The flowsheet (Chart VIII) describes the above reactions.

Chart VIII

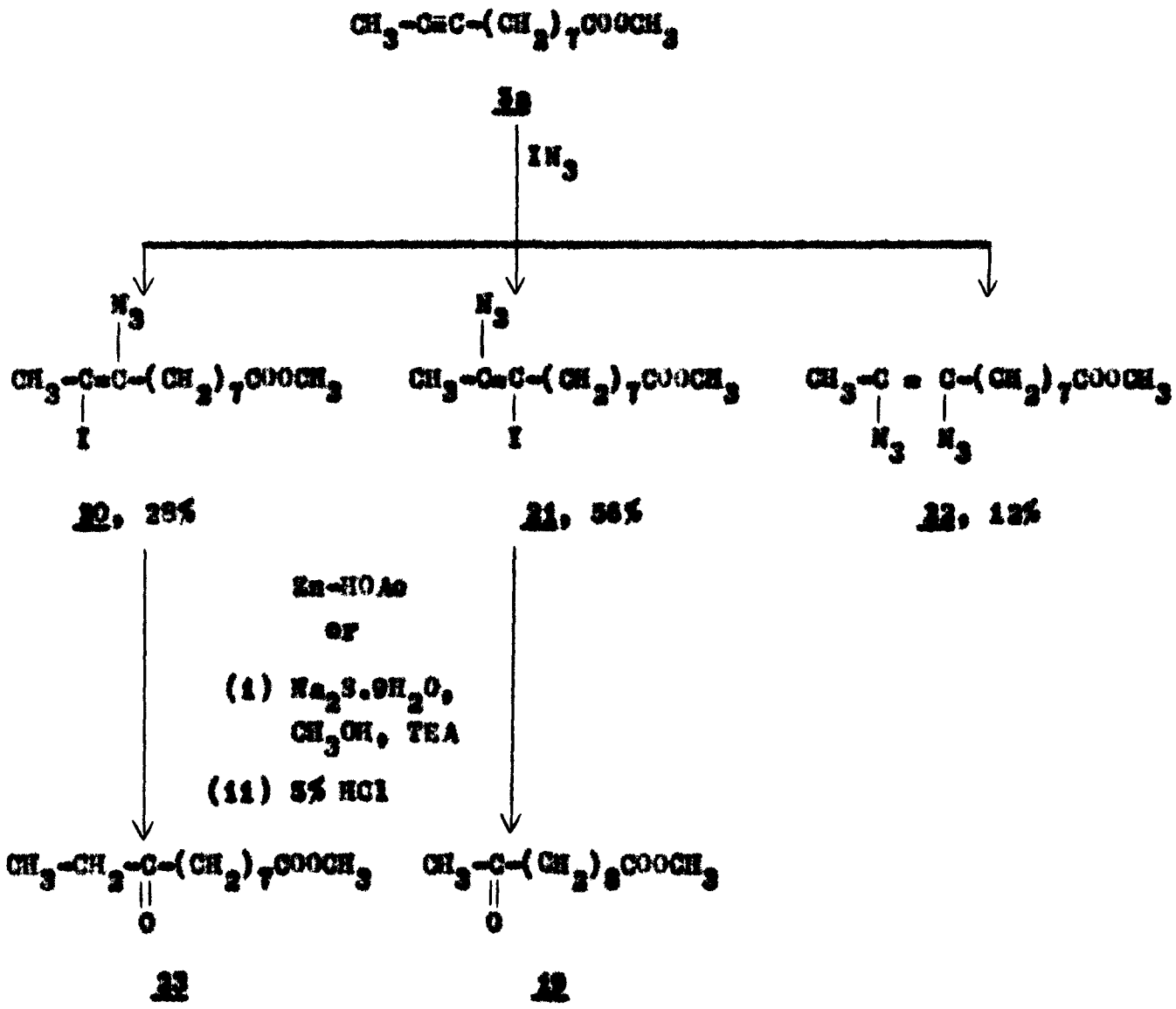


(b). Of Methyl 9-Undecynoate

Similar addition of IN_3 to methyl 9-undecynoate (5a) gave a separable isomeric mixture of methyl 9-azido-10-iodoundecenoate

(20, ca. 28%) and methyl 10-azido-9-iodoundecenoate (21, ca. 56%) along with a minor product namely, 9,10-diazidoundecenoate (22, ca. 12%). The reduction of 20 and 21 with zinc in acetic acid as well as by Na_2S method led to the formation of 9- and 10-oxoundecanoic (23 and 19) acids, respectively. The above reactions are outlined in Chart IX.

Chart IX



It is interesting to note that the addition of iodine azide to terminal acetylenic ester (3a) gave exclusively the regiospecific adduct 18 whereas separable regioisomers 20 and 21 were obtained from penultimate acetylenic ester (5a).

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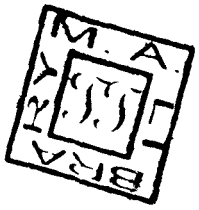

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Aligarh

This is to certify that the work embodied in this thesis entitled "Studies on Seed Fats and Fatty Acids" is the original work of Mr. Mukesh Babu done under my supervision. The thesis is suitable for submission for the award of the degree of Doctor of Philosophy in Chemistry.

Dr. S.M. Osman
Professor of Chemistry

Dedicated

to my mother

whose unfailing love

has always been a

source of

inspiration

to me

ACKNOWLEDGEMENTS

Words fall short in expressing my gratitude to Professor S.M. Osman whose constant guidance at every step has enabled me to accomplish this work.

My indebtedness to Professor M.S. Ahmad, can in no way be mitigated regarding the help which he has extended to me during various discussions concerning these researches.

I am also extremely grateful to Professor W. Rahman, Head, Department of Chemistry for providing me necessary laboratory facilities during completion of the work.

I also owe a great deal to Dr. Fasih Ahmad for his all-out efforts in construction of this thesis work. I also express my sincere appreciations to Drs. Moghisuddin Ahmad, M. Shamim Ahmad, Ishtiaque Ahmad, and other fellow researchers for their encouragement, co-operation and help. I am specially thankful to Mr. Mansoor Mahmood Siddiqui who strained himself in drawing the spectra besides helping in research work.

My acknowledgements are more due to Mr. I.A. Jilani for typing the script. Finally, I gladly acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, for financial assistance.

Mukesh Babu

C O N T E N T S

SUMMARY

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Part - I deals with the compositional studies on herbaceous seed oils.

Part - II concerns with the reactions of terminal and penultimate acetylenic fatty acids with perbenzoic acid, hypobromous acid, and iodine azide.

PART - I

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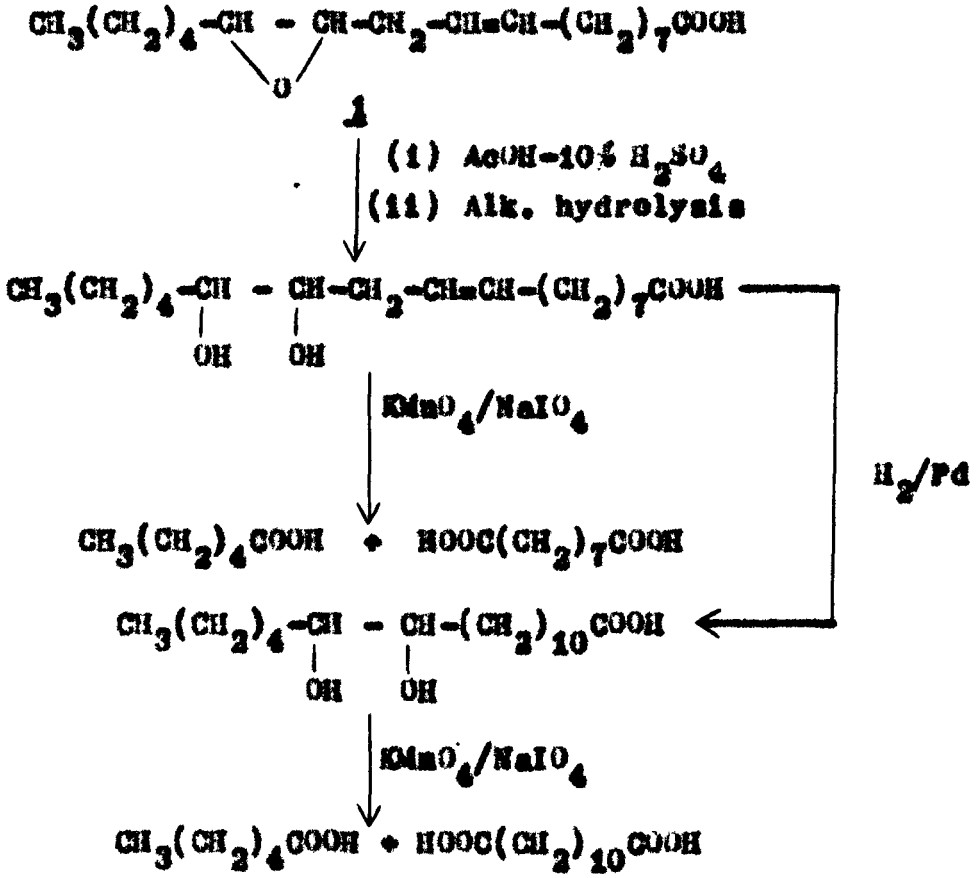
Durbetaki titration of Abutilon indicum seed oil showed it to contain HBr-reactive fatty acids (5%).

The concentrate of non-oxygenated fraction responded to Halphen test for the presence of cyclopropenoid fatty acids (CPFA). The cyclopropenoid acids were characterised as a mixture of

malvalic (2.3%) and sterculic (0.9%) acids along with normal fatty acids by the GLC of the silver nitrate-methanol treated methyl esters.

Successive crystallisations of the crude oxygenated fatty acid concentrate afforded threo-12,13-dihydroxy oleic acid. From this and other evidences it is concluded that 12,13-epoxyoleic (vernolic) acid (1) is present as a constituent (1.6%) of the glycerides. The chemical reactions outlined in Chart I were performed to establish the structure of vernolic acid.

Chart I



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active fatty acids in *A. indicum* seed oil is interesting and deserve further research in lipid biogenesis.

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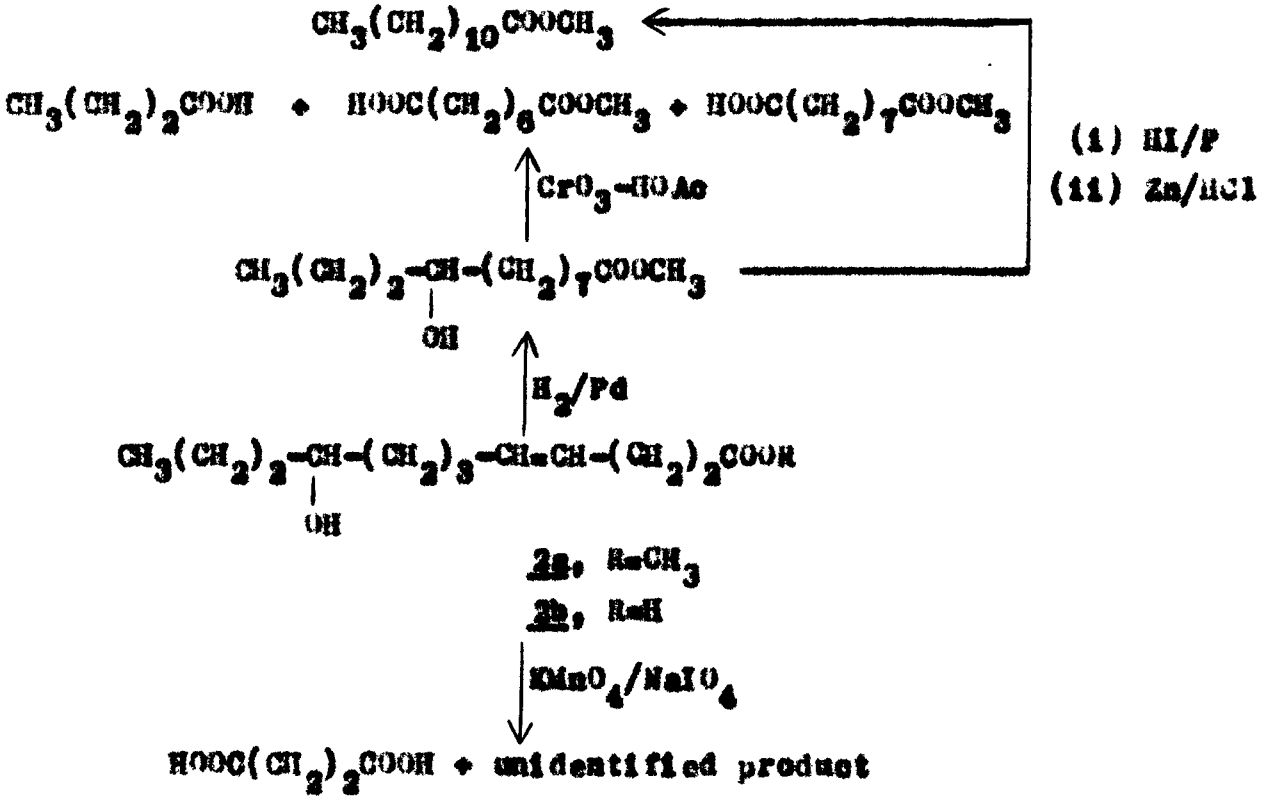
Seed oil of *Aniseochilus carnosus*, hitherto unexamined species, was found to contain a new short-chain monohydroxy unsaturated fatty acid (1.8%) in addition to the normal fatty acids. The hydroxy ester (2a) was isolated by column chromatography and characterised by its spectral behaviour and chemical transformations (Chart II) as methyl 9-hydroxy-*cis*-4-dodecenoate.

Chart II



PART - II

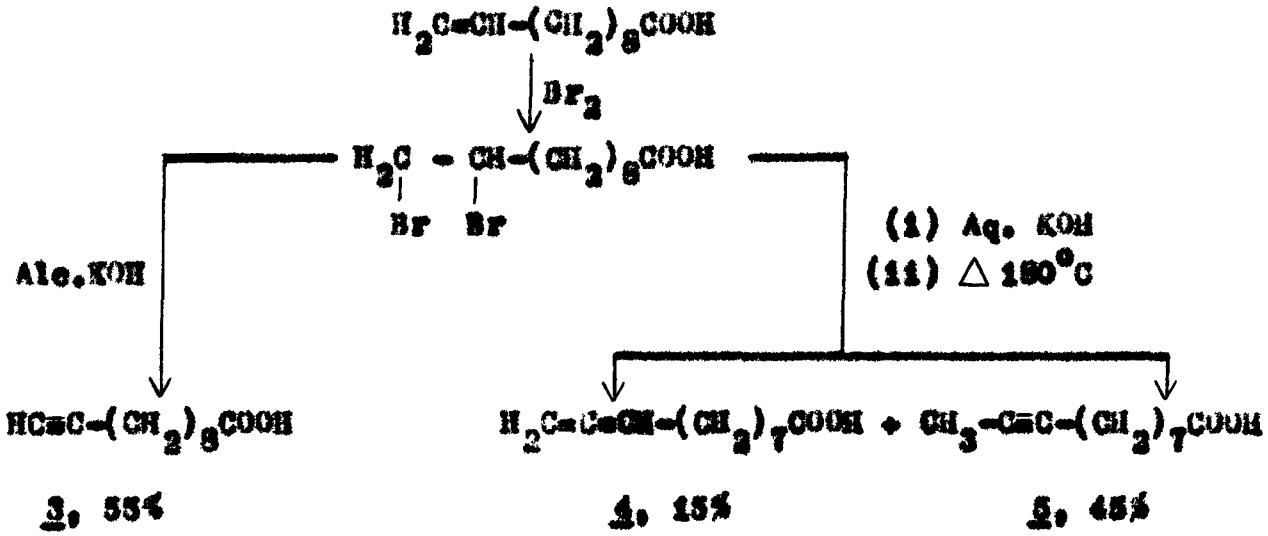
Reactions of Acetylenic Fatty Acids

As acetylenic acids have attracted less attention in the study of fatty acid chemistry, two acids, 10-undecynoic (3) and 9-undecynoic (5), have been prepared by the usual bromination-dehydrobromination method (Chart III).

1. 9,10-Undecadienoic Acid: A Co-Product During Synthesis of 9-undecynoic Acid

A co-product, hitherto unreported, obtained during acetylenic acid (5) synthesis (Chart III), was isolated and characterised as 9,10-undecadienoic acid (4) by combustion and spectral data.

Chart III



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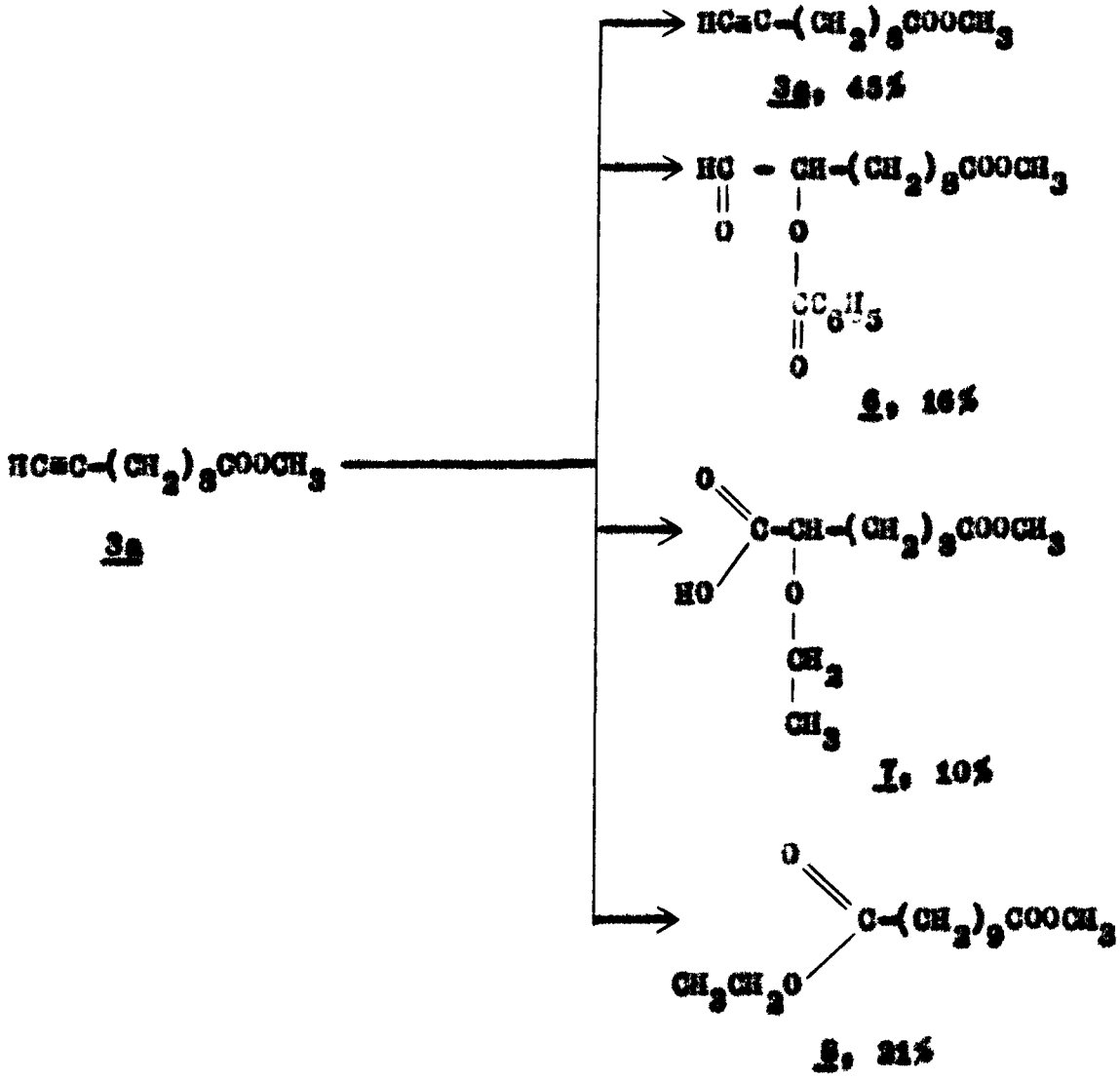
With an objective of preparing oxirene (oxacyclopropene) and/or dioxabicyclobutane derivatives, the perbenzoic acid (PBA) oxidations of methyl 9- and 10-undecynoate were investigated. Attempted epoxidation of the acetylenic esters resulted in the formation of rearranged and addition products, instead of yielding the anticipated epoxides. It was observed that the products of PBA oxidations of these esters can be most conveniently accounted for as arising from the oxirene intermediate.

PBA oxidation of methyl 10-undecynoate

The reaction of methyl 10-undecynoate (3a) with perbenzoic acid in chloroform gave unreacted component (3a, ca. 45%), methyl 11-aldehydo-10-benzoyloxyundecanoate (6, ca. 16%), half methyl ester of 10-ethoxyundecane-11,1-dioic acid (7, ca. 10%), and ethyl, methyl undecane-11,1-dioate (8, ca. 21%) following the path depicted in Chart IV.

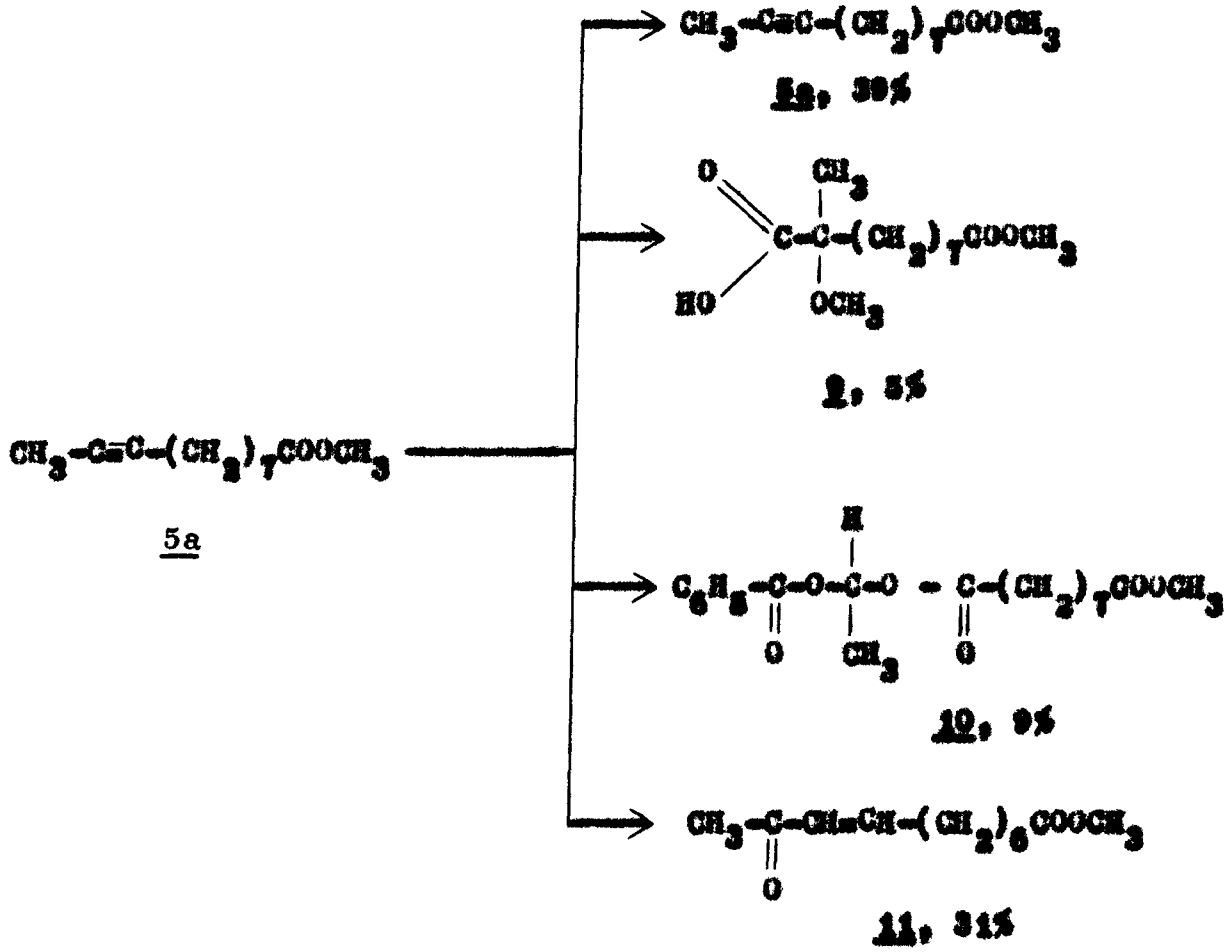
Chart IV


HC≡C-(CH2)8COOCH3
3a
HC≡C-(CH2)8COOCH3
3a, 45%
HC - CH-(CH2)8COOCH3
O O
CC6H5
O
8, 16%
O
C-CH-(CH2)8COOCH3
HO O
CH2
CH3
I, 10%
O
C-(CH2)9COOCH3
CH3CH2O
9, 21%

PBA Oxidation of methyl 9-undecynoate

Similar treatment of methyl 9-undecynoate (5a) with perbenzoic acid gave unreacted ester (5a, ca. 30%), half methyl ester of 9-methoxy-8-methylidenene-10,1-dioic acid (8, ca. 5%), methyl 8-ethylcarboxy(1'-benzoyloxy)octanoate (10, ca. 9%), and methyl 10-oxo-cis-8-undecenoate (11, ca. 31%). The above reaction is outlined in Chart V.

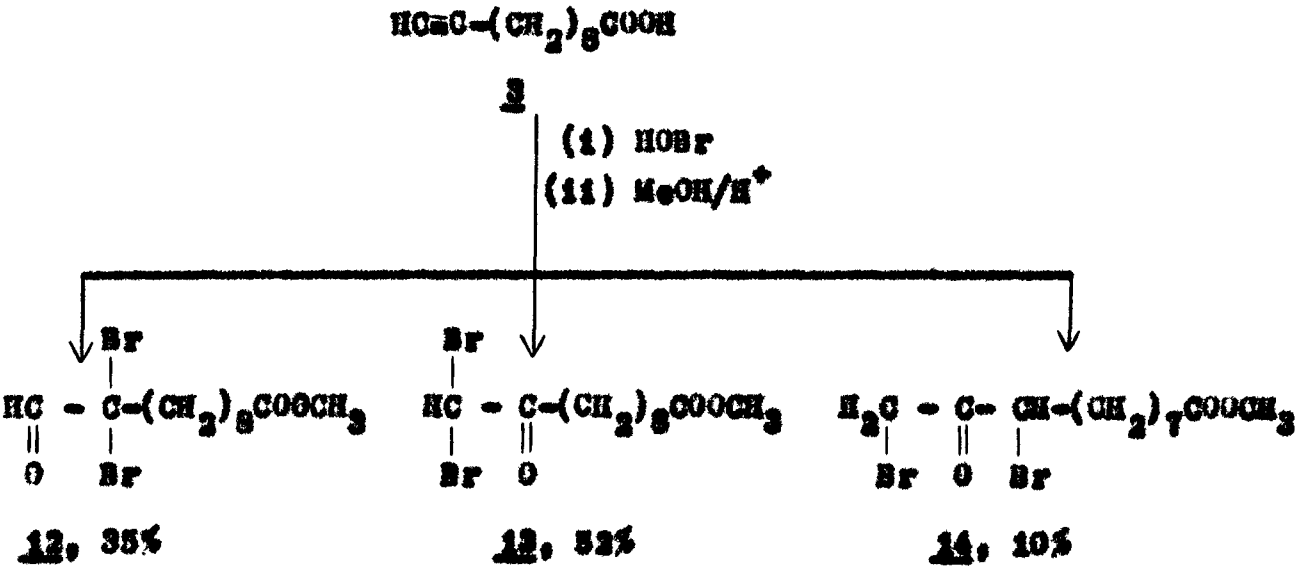
Chart V



3. Hypobromination of Acetylenic Fatty Acids

The reaction carried out in the present investigation (Chart VI) involves the addition of freshly prepared solution of hypobromous acid (HOBr) to 10-undecynoic acid (3). The resulting products after methylation yielded methyl 11-aldehyde-10,10-dibromoundecanoate (12, ca. 35%) and methyl 11,11-dibromo-10-oxoundecanoate (13, ca. 52%), and one minor component namely, methyl 9,11-dibromo-10-oxoundecanoate (14, ca. 10%). A mechanism is proposed to account for the formation of reaction products in relative yields.

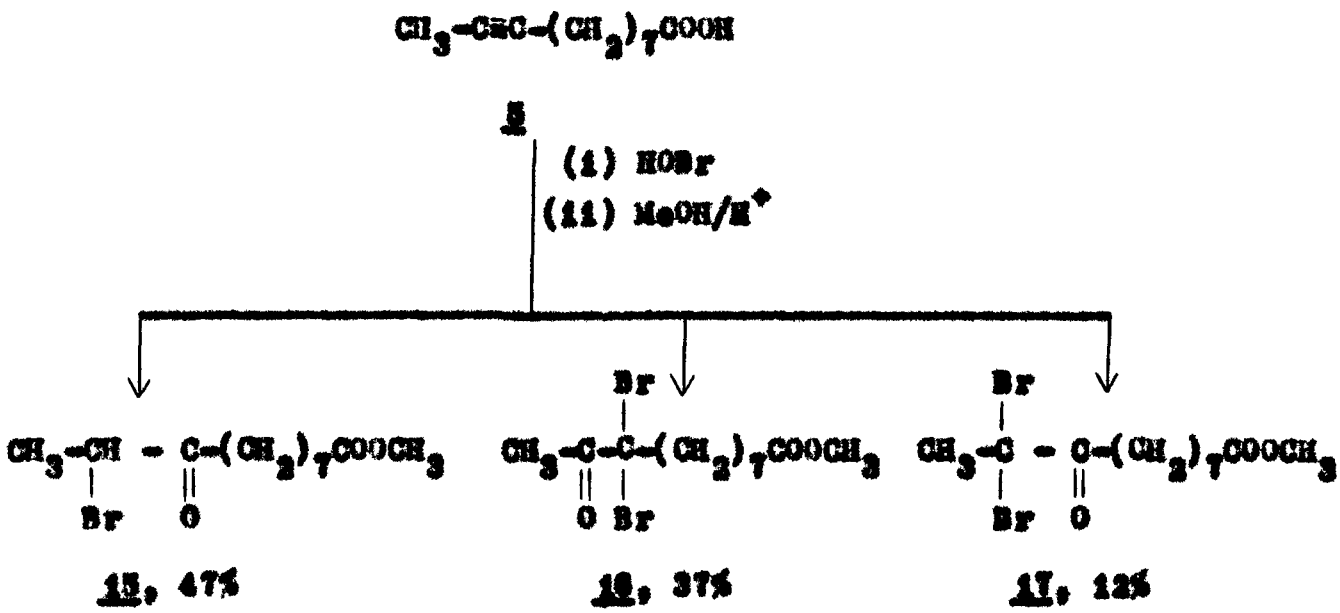
Chart VI



Similarly, the hypobromination of 9-undecynoic acid (5) followed by methylation (Chart VII) gave three products namely,

methyl 10-bromo-9-oxoundecanoate (15, ca. 47%), methyl 9,9-dibromo-10-oxoundecanoate (16, ca. 37%), and methyl 10,10-dibromo-9-oxoundecanoate (17, ca. 12%). To account for the formation of hypobromination products and their relative yields, a mechanism is also proposed.

Chart VII



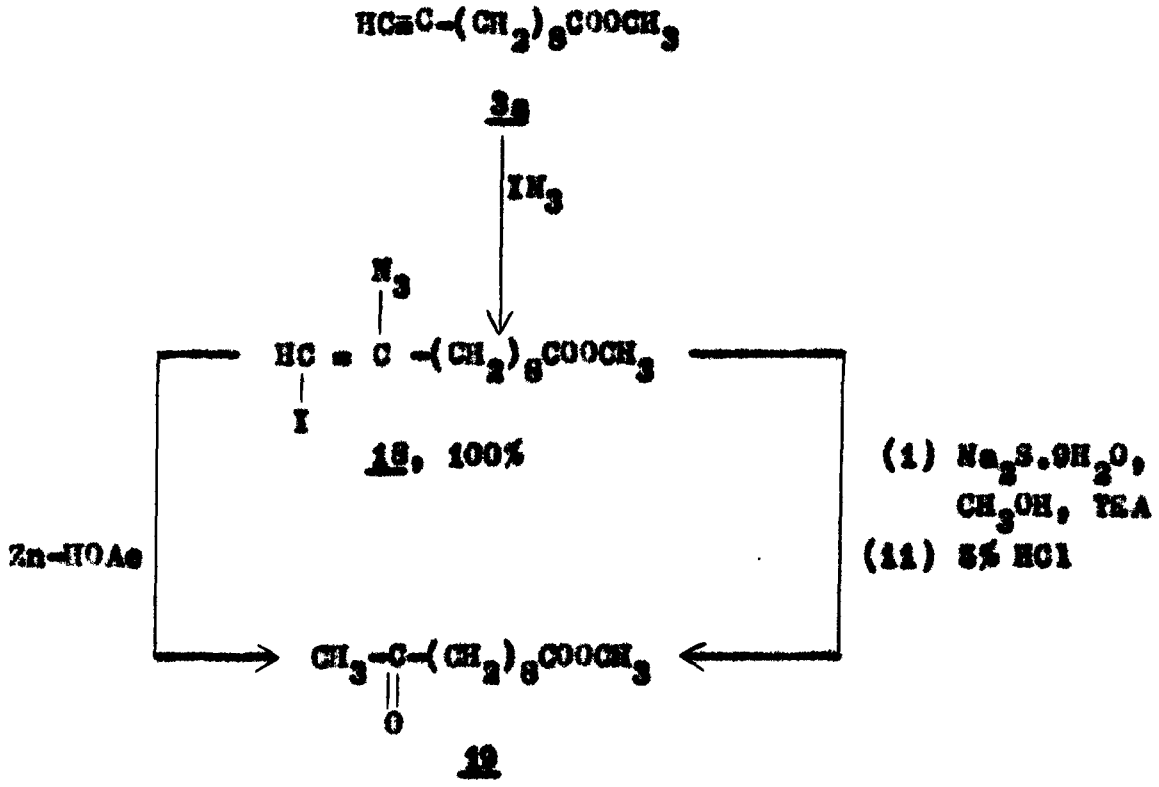
4. Preparation and Reactions of Vinyl β-Iodoamide Derivatives

(a). Of Methyl 10-Undecynoate

The addition of iodine azide (IN_3) to methyl 10-undecynoate (3a) was observed to proceed in a highly regiospecific manner. This addition led exclusively to the formation of 10-azido-11-iodoundecenoate (18) in nearly quantitative yield. The reaction of vinyl iodoazide (18) with zinc in aqueous acetic acid as well

as with sodium sulphide in methanol in presence of catalytic amount of triethylamine (TEA) furnished 10-oxoundecanoic acid (12) in 70 and 80% yield, respectively. The flowsheet (Chart VIII) describes the above reactions.

Chart VIII

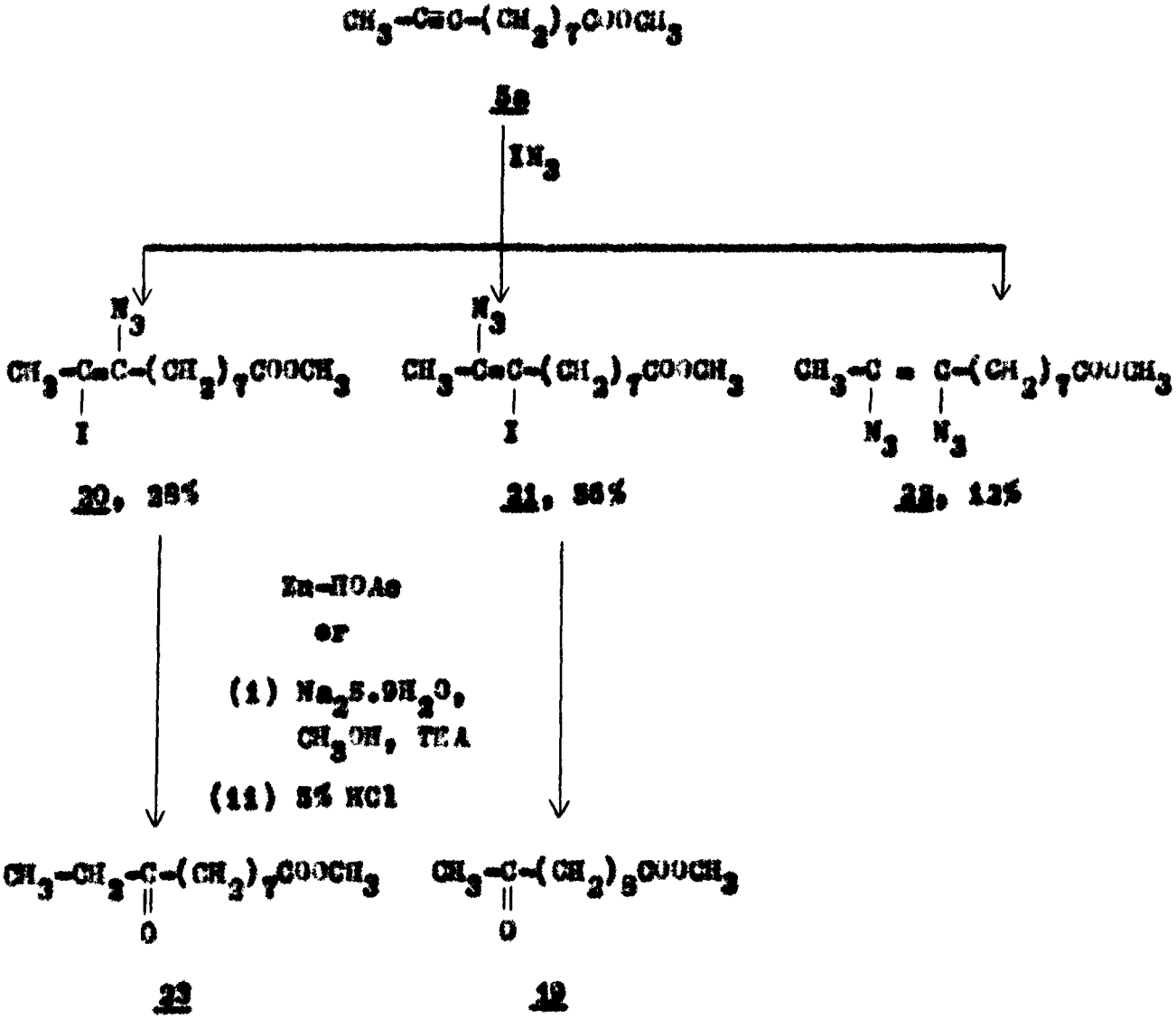


(b). Of Methyl 9-Undecynoate

Similar addition of IN_3 to methyl 9-undecynoate (9a) gave a separable isomeric mixture of methyl 9-azido-10-iodoundecenoate (20, ca. 38%) and methyl 10-azido-9-iodoundecenoate (21, ca. 56%) along with a minor product namely, 9,10-diazidoundecenoate

(22, ca. 12%). The reduction of 20 and 21 with zinc in acetic acid as well as by Na_2S method led to the formation of 9- and 10-oxoundecanoic (23 and 12) acids, respectively. The above reactions are outlined in Chart IX.

Chart IX



It is interesting to note that the addition of iodine azide to terminal acetylenic ester (3g) gave exclusively the regiospecific adduct 18 whereas separable regioisomers 20 and 21 were obtained from penultimate acetylenic ester (3g).

INTRODUCTION

The world of 1980's shall have to face the challenges posed by the rising cost of petrochemicals and availability of edible oils for the expanding population. According to Food and Agriculture Organisation (FAO) data, world output of oils and oilseeds is likely to fall in 1981. It is regrettable that although our country has achieved green revolution, the oilseed crops are cultivated largely under conditions of energy starvation. There is a vast potential of minor oilseeds which if properly tapped can substantially augment the overall supply of vegetable oils and can help in bridging the wide gap between their demand and supply.

As per world estimate the production of vegetable oils has grown exponentially over the last 20 years and has now reached about 35 million tonnes per year. This large increase in fat consumption is due mainly to the use of more vegetable fats in edible and non-edible oil industries. The vegetable oil industry in India today occupies a pivotal position in the mainstream of its economical development. It is estimated that our country's foreign exchange is to the tune of more than Rs.700 crores for importing oils and oilseeds.

The magnitude of the fats and oils industry amounts to one-fifth of the total petrochemicals industry. Vegetable oils contribute the greatest share to the industry. The phobia of heart disease has created a substantial shift towards the use of more vegetable oils as compared to hydrogenated fats. Further the rising cost of petrochemicals has forced the oil based industries to depend more on agrichemicals derived from agricultural products. The driving force for this new approach is that wild oil exouding plants are a renewable source of agrichemicals.

There has been in recent years a flurry of interest in the research of vegetable oils as a diesel fuel. More recently the importance of oils and fats is continuously increasing in the biochemical and biomedical sciences. Current researches in these fields are centred on the nutritional and physiological role of fats and the role of dietary fat in diseases, particularly cancer and coronary heart diseases. Considering all the above aspects it is apparent that the world of 1990's to face three major problems:

1. Newer sources of oils and fats both for edible and non-edible uses,
2. Interchangeability of oils and fats and demand for more PUFA containing hydrogenated oils, and

3. Production of proteins and agrichemicals from vegetable oils and oilseeds.

A research project of Indian Council of Agricultural Research (ICAR), New Delhi financed out of PL-480 funds has been in operation in Aligarh Muslim University since 1974. This project helped to explore the oilseed potential of little known wild uncultivated herbaceous species. The present work of compositional studies on fats and the chemistry of unusual fatty acids is directed to achieve the objective of finding alternative resources of oleochemicals and vegetable oils for the introduction of new oilseed crops.

P A R T – I

COMPOSITIONAL STUDIES ON HERBACEOUS SEED OILS

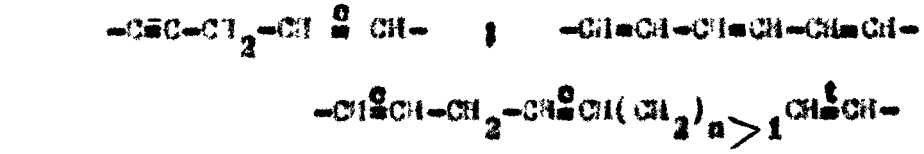
THEORETICAL

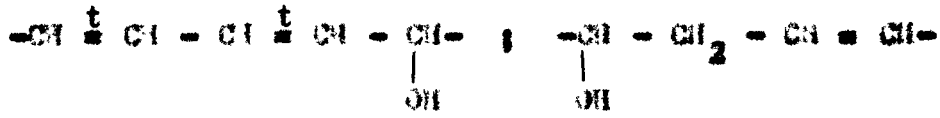
Vegetable oil is a major source of nutrition, especially for the vulnerable sections of society. The human body needs fats for energy and for storage as future food. The seed oils are also used for many industrial purposes apart from the important constituents of human diet. They are used as illuminants and for making soaps and lubricants, dressing of leather, yarn sizing, medicines, and cosmetics. The drying type of oils are used in the manufacture of surface coatings, oil cloth, and linoleum. The cakes left after removal of the oil are used as animal and poultry feed after suitable blending or as fertilisers. In the recent past, some of the minor seed oils have found a place in industrial food products such as chocolates. Application of these seed oils as an extender for cocoa butter in chocolates will give an impetus to these oils for export. There is a great demand for these products overseas since cocoa butter has become very expensive of late. Minor seed oils could be used in various other industrial products like fatty alcohols, fatty amines etc. after suitable processing. Currently, fats and oils possess about 40% of the paint binder, 50% of the surfactant, and 15% of the plasticiser markets. Other markets considered are: adhesives (1%), agrichemicals (10%), engineering thermoplastics (2%), and synthetic lubricants (20%).

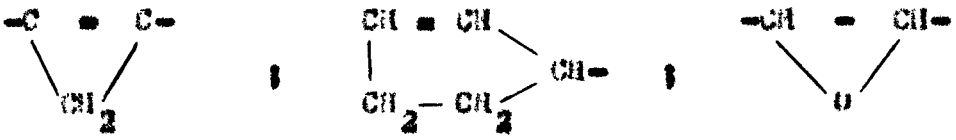
Natural Fatty Acids of Seed Oils

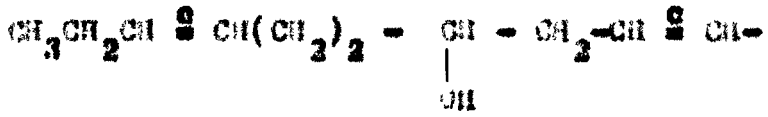
The long-chain fatty acids that are the subject of this thesis have their origins in wild vegetable seed oils. Natural fatty acids occur mainly as glycerides and a few as waxes. The make-up of the glycerides varies widely in composition as well as in fatty acid chain length and degree of unsaturation. More than 500 fatty acids have now been reported.

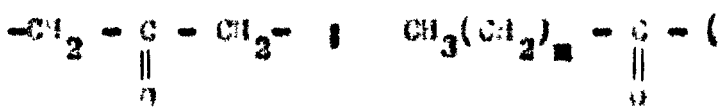
Natural fatty acids, whether saturated or unsaturated, are usually straight-chain compounds with an even number of carbon atoms and are most commonly C_{16}, C_{18}, C_{20}, or C_{22} compounds. The acids with branched chains, cyclic systems, and an odd number of carbon atoms are also known. Unsaturated acids have double bonds with usually *cis* (Z) configuration in certain preferred positions in the carbon chain. In addition, however, *trans* (E) olefinic, allenic, and acetylenic acids are known along with others that have conjugated unsaturation. Polyene acids have methylene interrupted unsaturation. Oxygenated functional groups of naturally occurring fatty acids include the hydroxyl, keto, and epoxy groups, of which the hydroxy-substituted acids are more common. Some unusual, naturally occurring fatty acids are those with the cyclopropene and cyclopentene structures. The unusual structural features of recently discovered natural fatty acids include the following groupings:

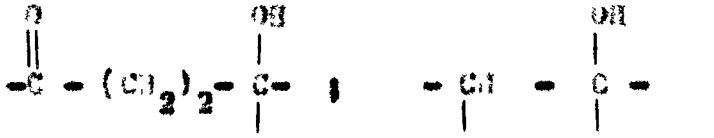


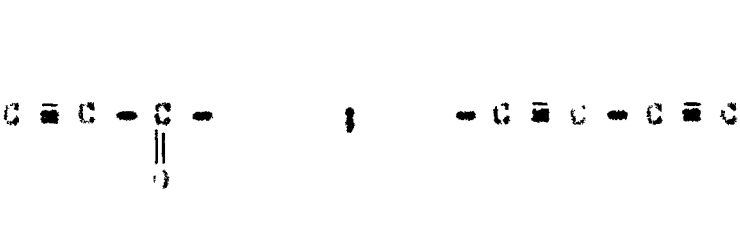












Besides the above structural groupings a large number of unfamiliar acids include unsaturated fatty acids with different chain length, position and geometry.

During germination and growth, different plants have many features of fatty acid metabolism in common. It is only during fruit and seed development that marked differences between species appear and the neutral lipids of seed oils are the source of more unusual fatty acid types.

Isolation and Characterisation of Fatty Acids

Physical and chemical methods for the analysis of oils and fats developed gradually over the nineteenth century, and are still quite widely in use for characterisation. Thus the saponification value to characterise mean molecular weight, the iodine value to measure unsaturation (including special technique to deal with conjugated systems), the refractive index as a check on unusual constituents, the hydroxyl or acetyl value, the titre value, and the hexabromide value all serve to indicate the type of fat that is to be handled[1-4]. The iodine value determinations by the Wijs and Hanus methods are standard procedures[1-4] adopted by many countries. Recently a rapid method[5] for the determination of iodine value has been developed in which a 2.5% solution of mercuric acetate in acetic acid is used as accelerator to shorten the reaction time. The colour tests for specific oils have been developed by observant chemists for qualitative analysis.

Next some methods of separation of fatty acids into various groups. Chromatographic methods have overruled the classical methods for separation of fatty acids. The tedious approach in fatty acid separation is the resolution of fats by countercurrent distribution (CCD) between immiscible solvents into a series of fractions that could be further analysed.

The first step in identification of an acid obtained from a seed oil is to obtain the acid in the pure state. Its melting point, ultraviolet (UV), and infrared (IR) spectra give clues to

the probable identity. If it is not possible to prepare a completely pure sample of the acid, it may be possible to prepare one or more pure derivatives from a concentrate. The characteristics to be determined then are type of functional group, chain length, number and type of unsaturation, and the positions of functional groups and that of the unsaturation.

The modern techniques of oil analysis include chromatographic, spectroscopic, and chemical methods.

(1) Chromatographic techniques

Three techniques dominate at present in the field of lipid analysis: thin-layer chromatography (TLC), gas-liquid chromatography (GLC), and latest innovated high pressure liquid chromatography (HPLC). Paper chromatography (PC) has not had much success in the lipid field.

Paper chromatography

After the introduction of TLC, the use of PC has become very limited. In this technique[6], the mixture of compounds is separated into the zones of different constituents on a strip of filter paper. The paper acts as a combination of partition, adsorption, and ion exchange. The paper is made to retain a stationary polar (dimethyl formamide or dimethyl formamide-H_2O) or a non-polar (paraffin, silicone etc.) phase and resolution is obtained by developing with a liquid immiscible with the fixed liquid. Reversed-phase PC is

preferred in most cases especially fatty acids, glycerides, sterols, and fatty alcohols.

Thin-layer chromatography

The principle of TLC was described as early as 1938. TLC gained recognition only from 1956 when Stahl[7] described equipment and procedures for the preparation of chromatoplates, and demonstrated the potential usefulness in the fractionation of substances. Adequate equipment such as spreaders for the uniform coating of glass plates and universally applicable adsorbents have become commercially available. The speed, sensitivity, and remarkable efficiency of separations (up to 50 mg of the sample) that can be achieved on layers of finally-divided solid materials, notably silica gel, coated on glass plates or even microscopic slides, using upward rising solvent systems has brought about a revolution in fat separation. The range has been further extended by the introduction of argentation or silver-ion TLC[8], which can resolve both by degree and type of unsaturation, and boric acid TLC[9] which is particularly good for the separation of various stereo (*threo* and *erythro*) forms of oxygenated materials.

Adsorption TLC has been used to separate non-oxygenated fatty acids, as a class, from epoxy acids, hydroxy acids, and dihydroxy acids.[10] The mixtures of esters of non-oxygenated long-chain fatty acids have been fractionated according to degree of unsaturation and chain length on layers impregnated with a

hydrophobic agent such as paraffins.[11] Cis- and trans isomers[12], and epoxy and hydroxy esters[13] have been separated by chromatography on silica gel G-silver nitrate. Recently silver sulphamate has been used in place of silver nitrate as argentation and charring reagent in TLC[14]. This reagent permits TLC separation of fatty acid esters with differing degrees of unsaturation as well as of cis/trans isomeric fatty acids, and allow for direct charring of fractions. Triglycerides have been fractionated on argentation[15] as well as boric acid[16] TLC. "Critical pairs" of acids or esters have been resolved by reversed-phase TLC after hydrogenation or bromination of the unsaturated partners,[11] bromination during reversed-phase partition TLC has also been reported[11]. Combinations of reversed-phase partition TLC and argentation TLC are found more useful for the separation of mixtures of saturated and unsaturated compounds.[17]

Separation of long-chain alcohols[18]; acetylenic and ethylenic materials[10]; epithio and epoxy fatty acids and their derivatives[19]; epoxy, hydroxy, halohydroxy and keto fatty acids[20]; mercapto acetic acid products of long-chain mono unsaturated compounds[21]; dicarboxylic acids[22]; polymers[23]; erepenynic and dehydroerepenynic acid esters[24]; bromo-derivatives of unsaturated fatty acids[25] were achieved by TLC.

Few diagnostic TLC spot tests using reagents, picric acid[26], Halphen reagent (5% sulphur in CS_2)[27], 4-(p-nitrobenzyl) pyridine[28], and 2,4-dinitrophenylhydrazine[29] have been developed for the

detection of epoxy, cyclopropene, acetylenic, and keto functions, respectively, in the mixed fatty acids of an unknown oil. An acidic and a neutral solution of 4,4'-bis (dimethylamino) diphenylcarbinol (BDC-OH)[30] can be used as a convenient and sensitive spray reagent for the detection of thiols and carboxylic acids, respectively.

Adulteration of valuable vegetable oils with animal fat or cheaper oil can be detected by TLC[31]. Lipids, after having been isolated by TLC, may be scrapped off the plates and extracted from the carrier material. TLC can be employed to monitor column chromatographic separation and course of reactions. Methyl esters from triglycerides were prepared on thin-layer chromatoplates[32]. A TLC method[33] has been developed for the class separation of plant neutral lipids. Utilising a two-step development in one dimension, lipid mixtures are separated into hydrocarbon waxes, steryl esters, methyl esters, triglycerides, fatty acids, diglycerides, sterols, and monoglycerides. The method[33] may be employed for quantitative and preparative purposes. A new technique for the complete separation of lipid classes in natural mixtures by two-dimensional chromatography[34] on thin-layers of silica gel impregnated with ammonium sulphate to improve separations has been introduced.

High performance thin-layer chromatography

In high performance TLC (HPTLC)[35] resolution per migration distance and per developing time is significantly enhanced compared with conventional TLC. The factors contributing to that enhancement

are: 1) superior resolution power of the layer, 2) adequate sample application, 3) controlled feeding of the mobile phase, and 4) control of gas phase influences. The merits of HPTLC are faster chromatography, more separation tracks per plate, better reproducibility, more sensitive, and better resolution. Precoated plates suitable for HPTLC are available in which adsorbents with smaller particle diameter (about 60 Å) and narrower particle size range than conventional plates are coated. These plates exhibit an uniform packing density in three dimensions and, as a consequence, solvent migration is significantly reduced and separation is improved as diffusion is significantly decreased.

Gas-liquid chromatography

Not too many years ago, the number of samples that could be analysed in the laboratory was limited principally by the speed of the analytical technique employed. However, with the advent of gas-liquid chromatographic (GLC) techniques, the number of analyses able to be performed increased greatly and became, in many cases, limited only by the speed at which the results could be processed. In recent years, use of the computer has tended to reverse this situation by its ability to process large volumes of data in extremely short times. Today, by combining GLC with computer data processing[36], a far greater number of analyses can be performed than ever before.

The technique dates back to 1952 when James and Martin[37] separated free fatty acids by this technique. Subsequently, the same authors[38] achieved improved separations using methyl esters. Although various chromatographic techniques are capable of fractionating fatty acid mixtures, direct quantitation is usually done by GLC using both polar and non-polar columns. A combined TLC and GLC technique can only permit a complete resolution and quantitation of all components of a mixture of fatty acids under investigation. Many biochemical and biological studies involve microgram samples for analyses and so GLC technique is of great value for such work. GLC technique is particularly useful for the identification of degradative fragments of a fatty acid.

In recent years, the use of preparative GLC has made possible the isolation of pure fractions from complex mixtures. The collected fractions can be chemically modified and then re-examined by chromatographic analysis. For example, keto compounds can be converted to N,N-dimethylhydrazides or reduced to hydroxy compounds; hydroxy esters can be oxidised to keto esters, acetylated or converted to the corresponding trimethylsilyl ethers, trifluoroacetyl, and isopropylidine derivatives.

High pressure liquid chromatography

High pressure liquid chromatography (HPLC)[39] is the latest innovation in the chain of chromatographic techniques which are used for the separation and identification of unknown compounds.

It has many advantages over conventional column chromatography. It is known for its high resolving power, speed and simplicity of the technique, suitability for automation, accuracy, precision and sensitivity in quantitative analysis, and effectiveness in qualitative analysis. This technique is merely an extension of the classical column technique, encompassing a detection system to indicate when the separated components elute off the column. Furthermore, the sample is not destroyed and fractions can be collected. Also the sample size requirements are small and usually a complete analysis can be carried out on a few micro litres sample. The chromatograms are reproducible with dependable retention times and accurate results.

Nevertheless HPLC has acquired prominence and popularity in lipid chemistry because this technique is not only as fast and almost as efficient as GLC but also many separations not possible by GLC can be achieved. No volatilisation or derivatisation into volatile derivatives as required for GLC is needed. Hence, molecular species originally present in the sample can be isolated. Since low temperatures are used, thermally labile compounds can also be separated. Like any chromatographic technique, HPLC can be used for separation, isolation, estimation, purification, and enrichment of lipids[40]. Positional and geometrical isomers of mono-,di-, and trienoic fatty acid methyl esters have been separated by silver nitrate HPLC[41]. Silver nitrate in the aqueous methanol phase[42] enabled the separation of the retention times within the

pairs oleic and elaidic, and erucic and brassidic acids. Separation of isomeric methyl hydroperoxyoctadecenoates and methyl hydroxystearates,[43] obtained by oxidation of methyl oleate, was achieved by HPLC. Triglycerides may be separated according to chain length and degree of unsaturation on silica HPLC columns.[44] Silica HPLC columns are generally considered useful for separation of classes of lipid compounds, whereas reversed-phase columns with octadecylsilyl or alkyl phenyl groups bonded to silica particles are used to separate homologous compounds found within a class.

(ii) Spectroscopic techniques

The spectroscopic techniques form a backbone of modern chemical investigations. They are used as routine analytical procedures for quality control in industries, for monitoring reactions, for probing structure of molecules, and for guidance in research and development. Four methods on electronic instrumentation viz., ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry (MS) are widely used. These methods are less time consuming and are often non-destructive.

Ultraviolet spectroscopy

The most important application of ultraviolet (UV) spectroscopy is the identification and estimation of various natural conjugated fatty acids in seed oils,[45] and most of the conjugated

acids of seed oils are of recent discovery. In an oil, intense absorption in the range of 200-300 nm (ϵ 10,000-20,000) signifies conjugation of at least two chromophoric groups. Intense absorption beyond 300 nm shows further conjugation. The acids[46] containing one double bond which is conjugated with the carboxyl group show absorption λmax near 208-210 nm and a lower ϵ_{max} than do the conjugated acids. The other important application[47] of UV is the estimation of polyenoic acids in oils and fats containing non-conjugated double bonds. The double bonds in these acids are rearranged to conjugated system by alkali isomerization. This method is now less frequently used since the advent of GLC.

Infrared spectroscopy

Infrared (IR) spectroscopy is one of the important physical methods used for the identification and estimation of various functional groups in oils and fats. One of the best known IR quantitative methods[48] in the chemistry of fats and oils is the determination of trans double bonds. The band at 970 cm^{-1} appears in IR spectra of all unsaturated compounds which contain a trans CH group. Other particular values in the detection of unusual functional groups are monosubstituted acetylene (2140-2100 cm^{-1}), disubstituted acetylene (2260-2190 cm^{-1}), allene (2222 and 1951 cm^{-1}), keto (1724 cm^{-1}), epoxy (945 and 825 cm^{-1}), hydroxy (3448 cm^{-1}), cyclopropenoid (1352 and 1010 cm^{-1}), furanoid (1572 cm^{-1}), cyano (2247-2222 cm^{-1}), and vinyl (990 and 909 cm^{-1}).

IR spectroscopy is widely used in the elucidation of structure of conjugated acids. Some of the important values for conjugated unsaturation are 952 cm^{-1} (enyne), 995 cm^{-1}(tt), 985 and 952 cm^{-1}(ct), none at 1000-950 cm^{-1}(cc), 987 and 958 cm^{-1}(ctt), 987 cm^{-1}(ttt), 991 and 931 cm^{-1} (ctc), and 993 and 950 cm^{-1}(ctte), and 993 cm^{-1}(tttt).

The use of NMR and MS spectroscopies in the structure determination of fatty acids is described in Part-II of the thesis.

(iii) Chemical methods

The chemical methods are to be employed in almost all the cases for an unambiguous characterisation of the fatty acids, inspite of the development of chromatographic and spectroscopic techniques. The chemical methods generally used, include the following standard organic reactions:

(a) Hydrogenation

(b) Hydroxylation

(c) Oxidative degradation

(d) Partial hydrogenation and partial oxidation

(e) Hydrogen bromide reaction

(f) Diels-Alder reaction

Besides the above reactions the following procedures have been found to be more useful for solving special type of structural problems:

(g) UBr-titration of oil before and after reduction by $LiAlH_4$ to distinguish between the epoxy and cycloprepenoid acids.

(h) Cleavage of saturated hydroxy acid by solid $KMnO_4$ for the location of hydroxyl group.

(i) Dehydration of a dienol to all trans triene acid by treatment with glacial acetic acid for establishing the presence of allylic hydroxyl group.

(j) Reduction of secondary alcoholic group $-CHOH-$ to $-CH_2-$ by hydriodic acid and phosphorus for determining the chain length.

(k) Reductive removal of hydroxyl group by the reduction of the tosylate with $LiAlH_4$ followed by oxidative degradation of unsaturated acid by permanganate-periodate for locating the position of unsaturation.

(l) Lipoxidase catalysed isomerisation to conjugated acids for detecting cis, cis-methylene interrupted double bonds in the unknown acid.

Recently several new methods have been developed for the identification of fatty acids. They include:

(m) A rapid method[49] for estimating fatty acids level in vegetable oils in which sample preparation including saponification, methylation, and extraction is carried out in a single tube prior to GLC analysis.

(n) A rapid, mild, and convenient transesterification of triglycerides using 0.2N methanolic (m-trifluoromethylphenyl) trimethylammonium hydroxide[30].

(o) Intramolecular oxymercuration[31] of alcohols derived from long-chain fatty acids having double bonds at 3t, 4c/t, or 5c/t which furnishes ethers.

(p) Ozonolysis[32] of unsaturated fatty acids in presence of BF_3-MeOH to give oxidative fission acid products as methyl esters for establishing the position of double bond.

(q) Reductive ozonolysis[33] of dienoic fatty acids to alcohols with sodium borohydride for the analysis of diene isomers.

Unusual Fatty Acids of Seed Oils

Fatty acids with features unrelated to usual structures exist naturally. They are often found only in the seed oils of related plants, and may be restricted to a few individual species, a genus, or a whole family; however, within this narrow distribution they may represent the principal acid present. The variety of unusual fatty acids in plants suggests that plants could be classified on the basis of qualitative or quantitative composition of their seed oils. A more complete tabulation of unusual fatty acids from plants was given by Smith[54] and this was updated later by Hitchcock[53] and by Smith[56]. Among the unusual fatty acids discovered in recent years, olefinic, Diels-Alder-reactive, and hydroxy acids will be discussed here.

(1) Olefinic fatty acids

A number of olefinic acids ranging in chain length from C_{14} to C_{23} and containing 1-4 double bonds have been reported in recent years. To cite the major features, Aphananthe aspera (Ulmaceae) seed kernel oil[57] was found to contain the highest levels (55.1%) of linoleic acid; and Koureopsis obliquifoliata (Connaraceae)[58] oil was found as new source of palmitoleic (cis-9-hexadecenoic) acid (32%), a relative rareness in plants. Spencer et al.[59] have reported the presence of 82% cis-6-hexadecenoic acid in Thunbergia alata (Acanthaceae) seed oil. cis-6-Octadecenoic acid has also been discovered in many species of Proteaceae.[60]

The 20:1 acid was first isolated by Hopkins[61] in 1940 from the seed oil of Conringia orientalis (Cruciferae) and was identified as the cis Δ-11 acid. The 22:1 (cis-13-docosenoic, erucic) acid is a major component of most Cruciferae seed oils[62]. However, several crucifer seed oils with no erucic acid have been reported. Instead they contain considerable proportion of linolenic, or eicosenoic acid[62,63] viz., Camelina sativa seed oil[64] was found to contain cis-11-eicosenoic acid with no erucic acid and the seed oils of Matthiola parviflora and Torularia torulosa with few other species[62,63] free from erucic were characterised by high levels of linolenic acid. Seed oils of the genus Limnanthes (Limnanthaceae) are unique in containing over 95% of the fatty acids with chain lengths greater than C_{18}, including 63% 20:1, 18% 22:1, and 13% 22:2.[65,66] The double bond is in 5 position in most of the 20:1 and a portion of the 22:1 acids. cis-5-Eicosenoic as well as cis-5-octadecenoic acids were identified as constituents of Thalictrum venulosum (Ranunculaceae)[67] seed oil. ω3 Monoenes ranging in chain length C_{14} to C_{28} and ω9 monoenes of C_{18} to C_{24} chain lengths have been found to occur in seed fat of Grevillea robusta (Proteaceae).[68]

The seed oil of Echium plantagineum (Boraginaceae)[69] has shown to contain two polyunsaturated fatty acids not commonly found in vegetable oils; all cis-6,9,12-octadecatrienoic acid and all cis-6,9,12,15-octadecatetraenoic acid. Polyunsaturated C_{20} and C_{22} acids were isolated from rapeseed oil[70]. The acids 20:2

and 20:3 were identified as all cis Δ-11,14, and all cis Δ-11, 14,17, and the acids 22:2 and 22:3 were characterised as all cis Δ-13,16 and all cis Δ-13,16,19.

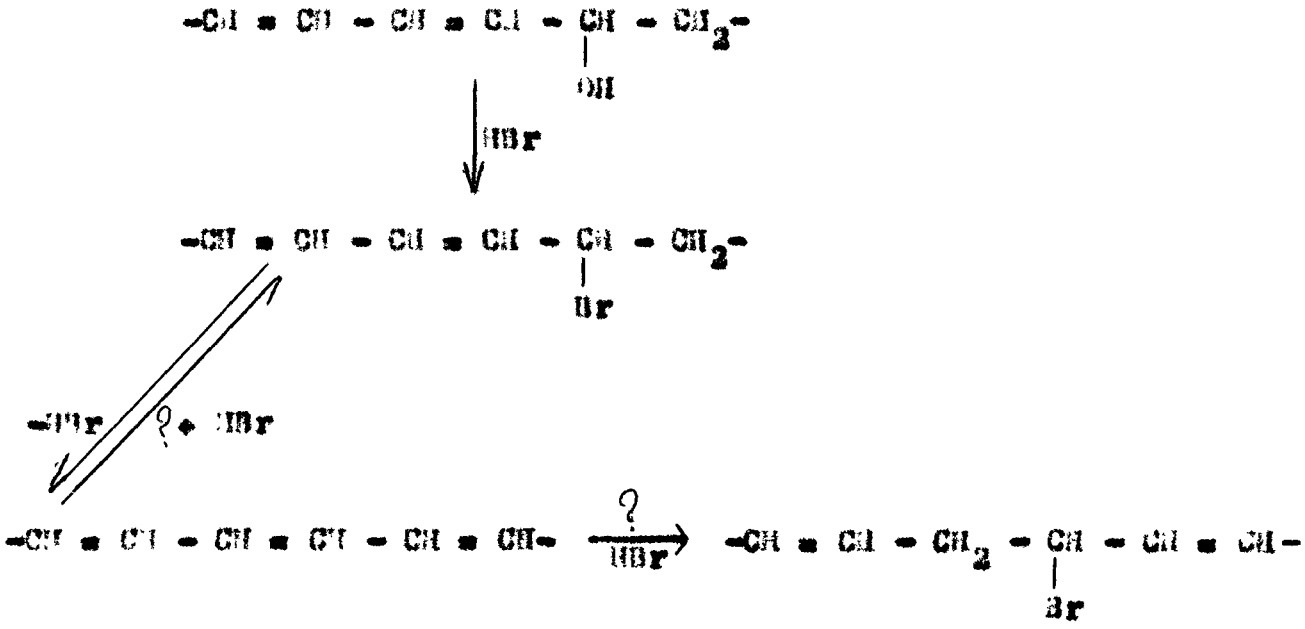
Fatty acids with conjugated unsaturation occur in many seed oils and constitute an interesting class of natural products. About 40 acids[71] with conjugated unsaturation have been identified in seed oils of 11 plant families ranging from the primitive Santalaceae to the relatively recent Compositae. It is evident that conjugated acids occur more or less at random and there seems to be no evidence of any pattern. Most of the conjugated acids of seed oils are of comparatively recent discovery.

Previous work[72,73] at Peoria laboratory has reported the analysis of a number of seed oils of Labiatae and found that the sub-family Stachyoideae is unique in the frequency with which allenic acid occurs. The first allenic acid was reported in Labiatae by Bagby et al.[72] to be (-)-5,6-octadecadienoic (laballenic) acid, and the second 8-hydroxy-5,6-octadecadienoic acid was isolated from Lamium sebiferum seed oil[74]. The third lamellenic acid was isolated from Lamium purpureum seed oil[75]. Recently seed oils of Leucas cephalotes[76] and L. urticifolia[77] have been reported from author's laboratory to be richest sources of laballenic acid.

(3) HBr-reactive fatty acids

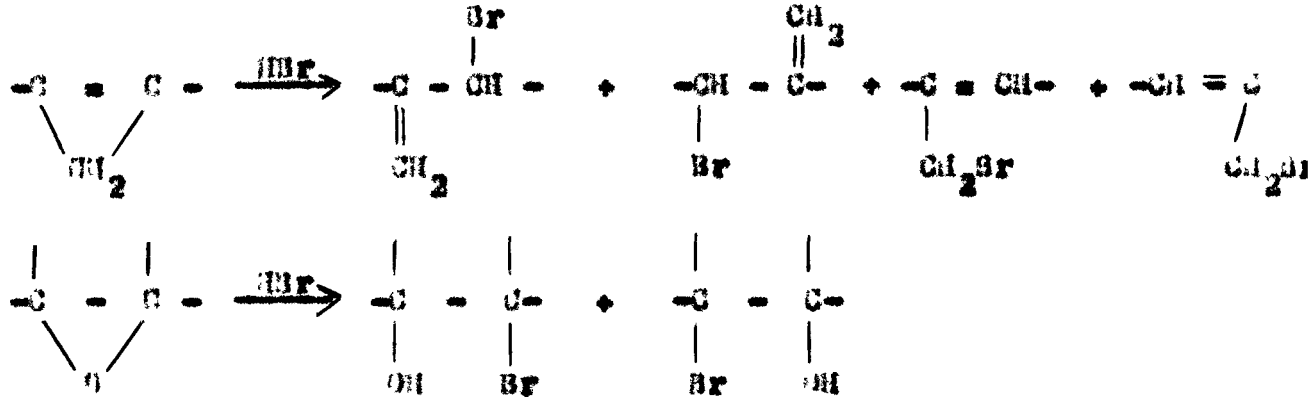
Hydrogenbromide (HBr)-titration in benzene-glacial acetic acid at ambient temperature lacks the specificity required when

the analysis is applied to a large variety of seed oils. Among the structures which absorb approximately stoichiometric amounts of HBr are the α-hydroxy conjugated diene grouping of dienol acids, the oxirane oxygen of epoxy acids, and the cyclopropene moiety of cyclopropenoid acids. Studies of dimorphecolic acid and of synthetic model compounds having the same functional groupings, carried out by Lohmar et al.[73] showed that initial uptake of HBr during rapid titration, in which 1 mole of HBr is taken up per mole of compound, was caused by a replacement reaction since essentially all the dienoid absorption is preserved immediately after titration.



Subsequently on standing, HBr is lost as triene is formed from a portion of the diene. The course of the reaction is not fully known, but possibly there is some re-addition of HBr as shown above.

During the titration, the reaction of HBr with cyclopropene ring involves the formation of four isomeric monounsaturated monobromo moieties and with epoxy group it gives two isomeric bromohydroxy structures, as shown below:



The structures of the reaction products have been investigated by Bailey *et al.*[79]

The American Oil Chemists' Society (AOCS)[1] and the International Union of Pure and Applied Chemistry (IUPAC)[4] procedures for determination of oxirane oxygen by titration at low temperature (5°C) permits the selective HBr-titration of epoxides in the presence of cyclopropenes and/or α-hydroxy dienes, the latter two types of groupings react with HBr[30a] at higher temperature (55°C). Titration at two different temperatures can quantitatively differentiate epoxide from the other HBr-reactive fatty acids. It has been realised that some cyclopropenoid material reacts with acetic acid solvent at 55°C during 30 minute titration time. To overcome this, Nosle *et al.*[31] demonstrated a modified method for the determination of total cyclopropenoid material by reaction with HBr in benzene

medium. Contrary to previous methods, this reaction proceeds rapidly at room temperature and obviate the undesirable need for the use of elevated temperatures in total cyclopropenoid determination.

The other procedure to determine oxirane oxygen in oils containing also cyclopropenoid acid involves utilisation of the HBr-titration before and after reduction by lithium aluminium hydride[82]. This reagent reduces epoxides, but has no effect on cyclopropenes or α-hydroxy conjugated dienes. Dienol acid may, if present, be determined quantitatively by UV spectroscopy, and cyclopropenes by Halphen colour test[83]. Preliminary evidence suggests that some seed lipids contain unsaponifiables which also react with significant amounts of HBr. An additional procedure for using halogen acids analytically to estimate epoxides and cyclopropenes involves stoichiometric addition of HCl followed by a quantitative determination of chlorine, with prior removal of epoxides, if present, by a pretreatment procedure. The three HBr-reactive acids are described below under the separate headings.

(a) Conjugated dienol acids

The unique α-hydroxy conjugated diene structure was shown to present in several members of the seed glycerides especially of the family Compositae and evidence for their wide occurrence in seed oils of the Compositae has been presented by Morris et al.[84]

Chisholm *et al.*[85] have found the conjugated *cis, trans* dienols as constituents of *Tragopogon porrifolius* (Compositae) seed oil. Dimorphecolic (9-hydroxy-*trans*-10,*trans*-12-octadecadienoic) acid was first isolated from the seed oil of *Dimorphotheca sinuata* (Compositae).[95] This acid represents 65-67% of the seed oil of this species[96], and also is the predominant fatty acid in oils from other *Dimorphotheca* species as well as certain species of the related genera *Osteospermum* and *Castalis*[97]. Dimorphecolic is the prototype of a class of fatty acids with a distinctive conjugated dienol grouping. Coriolic (13-hydroxy-*cis*-9,*trans*-11-octadecadienoic) acid,[98] discovered subsequently as a major constituent of *Coriaria napalensis* (Coriariaceae) seed oil, is a geometric and positional isomer of dimorphecolic acid. Minor proportions of dimorphecolic and coriolic acids, or their geometric isomers, occur in seed oils of numerous species of the plant family Compositae. These acids have an obvious structural relationship to the hydroperoxides derived from linoleic acid by the action of lipoxygenases or by autoxidation, but they are not artifacts of isolation. Dimorphecolic acid confers some unusual properties upon dimorphotheca oil that make it particularly versatile among vegetable oils in its chemical reactions. After suitable processing, dimorphotheca oil affords a drying oil comparable to the product from tung in its film-forming properties.[99]

Morris et al.[34] and Chisholm et al.[35] characterised independently a mixture of two isomeric acids, 9-hydroxy-10,12-octadecadienoic and 13-hydroxy-9,11-octadecadienoic acids in seed oils of various species of Compositae. It was shown that these acids are either cis, trans or trans, cis in configuration. Badami et al.[90] reported the presence of only the 9 isomer (9-hydroxy-trans-10,cis-12-octadecadienoic) in the seed oil of Calendula officinalis (Compositae). Powell et al.[91] reported the co-occurrence of 9-hydroxy-trans-10,cis-12-octadecadienoic and 13-hydroxy-cis-9,trans-11-octadecadienoic acids in the seed oil of Xeranthemum annum (Compositae). They were able to separate these two isomers by countercurrent distribution of the methyl esters. In general, dimorphecolic, coriolic, and the cis, trans dienols have the D configuration. Kleiman et al.[92] have reported the presence of two new conjugated dienols with $\Delta 3$ unsaturation: 9-hydroxy-trans-3,trans-10,cis-12-octadecatrienoic and 13-hydroxy-trans-3,cis-9,trans-11-octadecatrienoic acids.

(b) Cyclopropenoid acids

The 1,2-disubstituted cyclopropene function occurs in the fatty acid chain of lipids from certain plants belonging to the order Malvales (Malvaceae, Sterculiaceae, Tiliaceae, and Bombacaceae families),[54,93] cotton seed oil being the most common. In 1952, Nunn first isolated the cyclopropenoid fatty acid (CPFA) from Sterculia foetida (Sterculiaceae)[94] oil and named sterculic

(9,10-methylenoctadec-9-enoic) acid. One of these fatty acids, malvalic (8,9-methylenoheptadec-8-enoic) acid is a component of cottonseed oil triglycerides[95]. Malvalic acid was first isolated and characterised by MacFarlane et al.[96] and recognised as a homologue of sterculic acid. Both the acids, viz., sterculic and malvalic often occur together and sometimes may be accompanied by small amounts of their dihydroderivative. In general, the quantity of sterculic acid predominates in Sterculiaceae and that of malvalic acid predominates in Malvaceae seed oils.

Two other cyclopropenoid acids have been discovered; D-2-hydroxysterculic acid in the seed oil of Pachira insignis (Bombacaceae)[27] and sterculynic (8,9-methylenoctadec-8-ene-17-ynoic) acid in the seed oil of Sterculia alata (Sterculiaceae).[97] Over a period of years, other investigators have found in various species indications of elusive cyclopropenes with chain lengths shorter than malvalic acid. Raju and Reiser[98] reported evidence for a CPFA with a GLC retention time shorter than malvalic acid in Althaea rosea (Malvaceae) seed oil. Johnson et al.[99] hinted the occurrence of C_{17} cyclopropenes in the fruits of certain Malva species. Ackman and Hooper[100] encountered an unusual component in Euphoria longana (Sapindaceae) seed oil which they regarded as possibly being a C_{11} CPFA with a cyclopropene function near the methyl end. Among the recent and comprehensive additions to the literature of CPFA has been those of Madrigal et al.[101] and according to them Pavonia sepium seed oil is unusual in being the

first of the family Malvaceae in which the content of sterculic acid was observed to be greater than that of malvalic acid disobeying the general trend of the family. In addition the seed oil of Pterospermum acerifolium (Sterculiaceae)[102] is also unusual in containing the malvalic acid in greater quantity than that of sterculic acid. More recently, Ahmad et al.[103] from author's laboratory reported Grioleana hookeriana, the second unusual seed oil of the family Sterculiaceae in which they observed the content of malvalic acid to be greater than that of sterculic acid.

Dihydrosterculic (cis-9,10-methylenecctadecanoic) acid was found in the seed oil of Euphoria longana (Sapindaceae).[104] Liu Kenlie et al.[105] discovered the co-occurrence of dihydrosterculic and cis-9,10-methylenehexadecanoic acids in lychee (Litchi sinensis, F. Sapindaceae) seed oil. An investigation of the seed oil of Byrsocarpus coccineus (Connaraceae)[106] disclosed the presence of lactobacillic (cis-11,12-methylenecctadecanoic) acid in its oil. Lactobacillic acid has long been known as a constituent of certain bacterial lipids, but this is the first report of its presence in a seed oil.

The CPFA containing seed oils are known to give a positive red colour in the Halphen test[83]. Zahorsky et al.[107] established the structure of the compounds responsible for the development of the red colour on reaction of CPFA with Halphen reagent (1% solution of sulphur in CS_2). The spectroscopic methods, particularly the IR and NMR spectroscopies are useful in the detection

and identification of CPFA. In IR spectrum CPFA showed two distinctive prominent bands at 1008-1010 and 1852 cm^{-1}, attributed to the in-plane wagging vibration of the ring methylene group and the stretching frequency of the ring double bond, respectively. Measurement of the absorption of 1008-1010 cm^{-1} has been suggested as a means of estimating the total CPFA content of a seed oil. In PMR spectrum the cyclopropene ring methylene protons give rise to distinctive signal at δ 0.88 (singlet).

It is observed that CPFA autoxidise and polymerise readily in air with opening of the cyclopropene ring at room temperature, and even slowly at 0°C, as indicated by the increase of the equivalent weight with time. To date no method has been described which can be used for the quantitative isolation of different CPFA in a mixture. It is possible, however, by selecting suitable seed oils as starting materials to obtain individual CPFA of high purity. Nunn[94] isolated sterculic acid of sufficient purity for structure determination by a combination of urea fractionation and low temperature fractional crystallisation of the fatty acids of Sterculia foetida oil. In 1963 Fogerty et al.[108] used liquid-liquid partition chromatography to obtain pure methyl malvalate from a concentrate of the ester prepared by the urea fractionation technique from S. foetida esters or from Gossypium hirsutum (cottonseed) esters. Nevens and Hopkins[97] used countercurrent distribution technique to obtain pure sterculynic acid, but this technique does not appear to have been tried for other cyclopropenoid acids.

The quantitation of CPFA is done by GLC and HBr-titration. It is evident from earlier investigations that the seed oils containing such an array of compounds are difficult to quantitatively analyse by traditional GLC and HBr-titration procedures. Kecourt et al.[109] have shown that CPFA tend to isomerise and decompose as they pass through the GLC column. In addition, GLC data show that the malvalic acid peak is masked by the linoleic acid peak[110] and that the corresponding cyclopropane acid may also be obscured by the presence of oleic acid[111]. Therefore the chemical modification of CPFA is required prior to GLC. The chemical reaction involves hydrogenation or reaction with mercaptans or with silver nitrate.

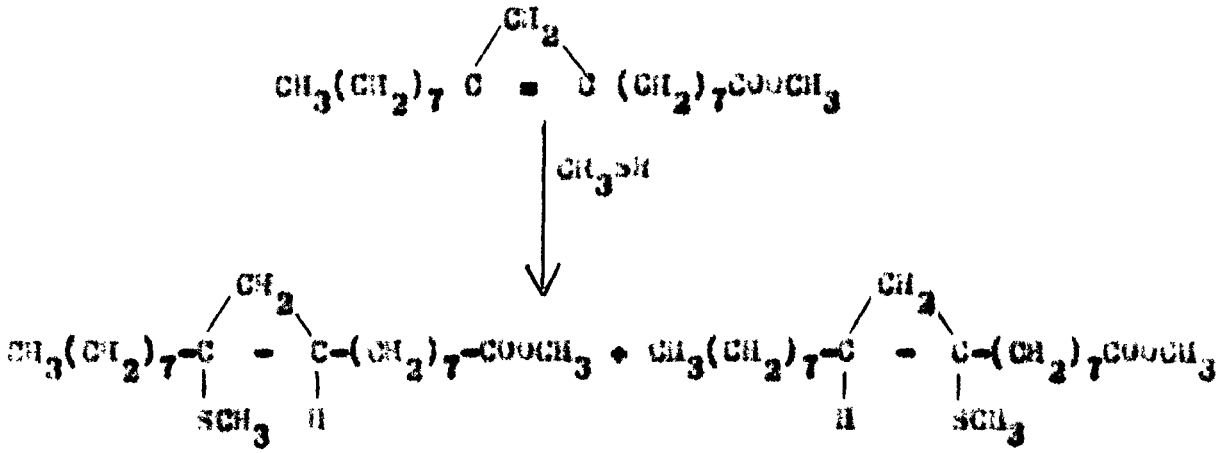
(i) Hydrogenation

Individual cyclopropenoid esters in a seed oil can be estimated by GLC analysis of the stable cyclopropane and branched-chain esters obtained by hydrogenation,[110] after a preliminary analysis of the unmodified mixture to determine other unsaturated components. Hilwa[111] has reviewed the complexity of such systems particularly when malvalic acid co-occurs with large amount of sterculic acid. Gellerman et al.[112] reported that Lindlar's catalyst (lead/quinolene poisoned palladium) which is commonly used to reduce acetylenes to olefins, reduces cyclopropenes to cyclopropanes with no over-reduction and without affecting any normal olefinic compounds which may be present. The resulting cyclopropane acid can be isolated, if necessary, for its structure determination by appropriate technique.

(11) Methyl mercaptan derivatives

The hydrogenation process often gives rise to a number of side products, unless conducted under very closely controlled conditions. Also the GLC of the hydrogenated product does not allow the analysis of the other unsaturated fatty acids present in the oil. The reaction of mercaptan with cyclopropene moiety has been performed first by Kircher[113] and this reaction suggested a new approach to the analysis of CPFA by GLC. This method estimates individual CPFA as well as normal and cyclopropene acids.

Mercaptans add readily across the cyclic double bonds in a CPFA to give two unresolved isomeric mercapto esters. The exact nature of the reaction is not known. The anticipated course of the reaction involves either a free radical or nucleophilic addition, as shown below:



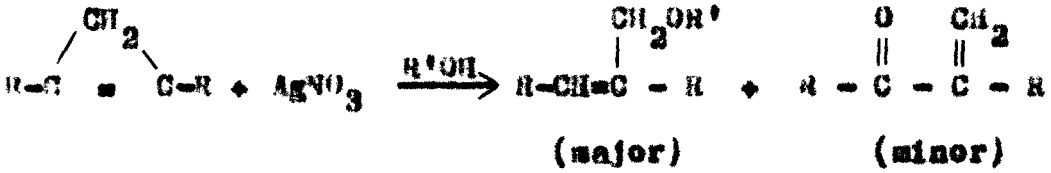
The absence of any additional peaks on gas chromatogram indicates that no side reaction occurs. However, if the reaction is carried out above 35°C, methyl linoleate also forms the additional product

which gives a peak in between the malvalate and sterculate derivatives. Other fatty acids are not affected by the reagent and can be estimated simultaneously from the same chromatogram.

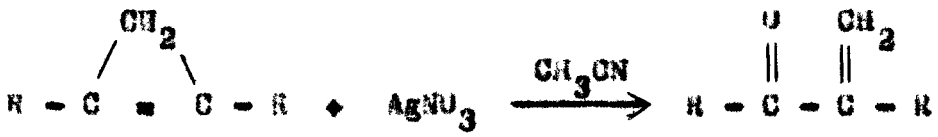
The methanemercapto adducts showed no sign of decomposition on GLC column up to 240°C and have longer retention times than normal fatty acid esters due to the presence of $-SCH_3$ group. So the individual cyclopropenoid components can be separated and estimated by this procedure.

(iii) Silver nitrate derivatives

In 1965 Kircher[114] investigated the reaction of sterculene (1,2-di-n-octylcyclopropene) with $AgNO_3$. The reaction is rapid in alcohols and the product is largely an alkoxymethylolefin with smaller amount of an α,β-unsaturated ketone.



In non-hydroxylic solvents (such as acetonitrile, acetone), the reaction is slower and an α,β-unsaturated ketone is the only product observed.



The utility of this reaction for the quantitation of CPFA in Sterculia foetida oil has been reported by Kircher.[114] Cyclopropane or other unsaturated components present initially are unaffected and can be isolated and determined separately.

Schneider et al.[115] reported that this method is applicable to oils containing from 0.01 to 100% of cyclopropenoid fatty acids. The derivatives of oils containing low levels of cyclopropenoids are separated from the normal methyl esters by alumina chromatography prior to GLC.

Theories on the biosynthesis of cyclopropenoid fatty acids can be summarized as follows:

Oleic acid + $^{\cdot}CH_2$ ⟶ dihydrosterculic acid ⟶ dihydromalvalic acid

Stearolic acid + $^{\cdot}CH_2$ ⟶ sterculic acid ⟶ malvalic acid

C_{17} monounsaturated acid + $^{\cdot}CH_2$ ⟶ dihydromalvalic acid

Incubation studies[99] employing several ^{14}C compounds indicate that the methyl group of methionine is the most likely precursor of the ring-methylene carbon.

These cyclopropenoid fatty acids have recently been the subject of intense investigation and are held responsible for numerous physiological disorders in farm and laboratory animals[116,117] and co-carcinogenic properties.[118] CPFA inhibit fatty acid desaturation in several species of animals causing the stearate-to-oleate ratio to rise. These acids may influence the composition

and function of membrane structures in animals,[119] since variations in lipid composition are known to alter permeability of membrane systems. Other biological effects observed in several species of animals include retarded growth in rats and chicks, delayed sexual development in female rats and pink discolouration to avian egg whites.[120]

The cyclopropane acids are more commonly found in micro-organisms than in plant sources. The function of these acids remains a matter of speculation and has been postulated to protect the phospholipids at a time when resynthesis would be difficult owing to sluggish metabolism.[121] Another view proposed is that the cyclopropane ring may be formed to preserve the configuration around the double bond rendering the hydrocarbon chain less susceptible to oxidation than is the double bond.[122]

(c) Epoxy acids

Fatty acids with epoxy groups occur naturally in seed oils of a considerable number of plant species. Not counting enantiomeric forms and excluding some additional epoxy acids in cutins, one C_{20} and nine C_{18} *cis* epoxy acids have been isolated from seed lipids.[92,123-125] The first seed oil of this group to be investigated was that of *Vernonia anthelmintica* (Compositae) in which Gunstone[126] discovered and characterised vernolic (*cis*-12,13-epoxy-9-octadecenoic) acid. This vernolic acid is the most familiar and widely distributed of the natural epoxy fatty acids.

Its isomer coronaric (*cis*-9,10-epoxy-12-octadecenoic) acid has been described as a component of *Chrysanthemum coronarium* (Compositae) seed oil.[127] *Stokesia laevis*,[128] another member of Compositae, produces an oil containing vernolic acid. Krewson and Scott[129] reported the presence of vernolic acid in *Euphorbia lagascae* (Euphorbiaceae) seed oil. These two (vernolic and coronaric) acids are obviously structurally related to linoleic acid. Epoxy acids structurally similar to linolenic acid and oleic acid have also been registered. An example of the former, *cis*-15,16-epoxy-9,12-octadecadienoic acid, occurs in *Camelina sativa* (Cruciferae) seed oil,[130] and the latter, *cis*-9,10-epoxystearic acid comprises 28% of the mixed acids from the uredospores of a wheat stem rust.[131] Recently Kleiman *et al.*[125] discovered and characterised alchornic [(+) *cis*-14,15-epoxy-*cis*-11-eicosenoic] acid, a C_{20} homologue of vernolic acid, in the seed oil of *Alchornea cordifolia* (Euphorbiaceae).

Although only one epoxy acid is found in some oils, in others two or more are present, e.g., *Helichrysum bracteatum* (Compositae) seed oil[123] was found to contain three epoxy components, coronaric acid, *cis*-9,10-epoxyoctadecanoic acid, and an acetylenic epoxide identified as *cis*-9,10-epoxy-12-octadecynoic acid; *Xeranthemum annum* (Compositae) seed oil[132] contains *cis*-9,10-epoxyoctadecanoic, coronaric, and vernolic acids. Kleiman *et al.*[12] have reported the previously unknown epoxy acid, *cis*-9,10-epoxy-*trans*-3,*cis*-12-octadecadienoic acid in *Stanleyanium macrocephalum*

(Compositae) seed oil. The seed oil of Crepis conyzaefolia (Compositae)[133] was found to contain previously unidentified cis-12,13-epoxyoctadec-trans-6,cis-9-octadecadienoic (14%) and cis-12,13-epoxy-cis-6,cis-9-octadecadienoic (2%) acids and the more common vernolic (32%) acid. All the naturally occurring epoxy acids discovered so far appear to be optically active.

Qualitatively, the compounds with epoxy groups are revealed by on-the-plate test with picric acid,[26] whereas the quantitative determination is being made by titration[80a] with HBr at near 0°C and the results are expressed as hydrogen bromide equivalent (HBE) and calculated as epoxyoleic acid. Now-a-days NMR technique[80b] is also used to determine the oxirane oxygen content. The suitable conditions for determination are 0.1 g epoxidised oil sample, 0.5 ml deuterated chloroform and 2 drops of tetramethylsilane. This method is simple, sensitive, and reproducible.

The spectroscopic techniques viz., IR, NMR, and mass are helpful in ascertaining the structure of even the small amount of epoxy acids present in an oil. GLC is also a powerful tool for the detection and estimation of epoxy components in seed oils. The use of GC-MS gives a very powerful method for studies of epoxy esters. The GLC behaviour of the cis and trans epoxy esters is summarised[134] and it has been concluded that the equivalent chain length (ECL) values obtained on the Apiezon L grease (APL) column are fairly reproducible but those on the diethylene glycol succinate(DEGS) column show more variations. Further, Gunstone[134] observed that

although most of the epoxy esters gave single peak on GLC while some gave more complex results indicating partial or complete decomposition of the epoxide. The quantitation of the epoxyoleate in an oil can be done by the use of added epoxy ester as an internal standard.

Toomi *et al.*[20] have separated the epoxy acids, esters, and alcohols by direct and reversed-phase TLC. In the TLC *cis* epoxy compounds can be easily separated from their *trans* isomers. The monoepoxy has a higher mobility than the corresponding diepoxy compound. Positional isomers of the same carbon chain lengths are separable (*cis*-6,7- from *cis*-9,10-), and also epoxy compounds of different chain lengths (C_{18} from C_{22}). By reversed-phase TLC separations are also possible on the basis of both the chain length and the number of epoxy groups in the chain. It should be noted here that the *cis*-9,10-epoxyoctadec-12-enoic acid and ester have the same R_f values,[135] respectively, as *cis*-9,10-epoxystearic acid and ester. These compounds are inseparable by TLC even after converting them to the chlorohydrins but can be distinguished by other methods (*vide infra*). In general, the chromatograms are developed with hexane-ethyl ether-acetic acid (80:20:1, v/v), and the spots are made visible with iodine vapours.

Two procedures are followed in preparing methyl esters from the epoxy oils. If the esters are meant for GLC only, the BF_3-methanol procedure[136] is selected. If separation and characterisation of esters are intended, the oils are first treated with

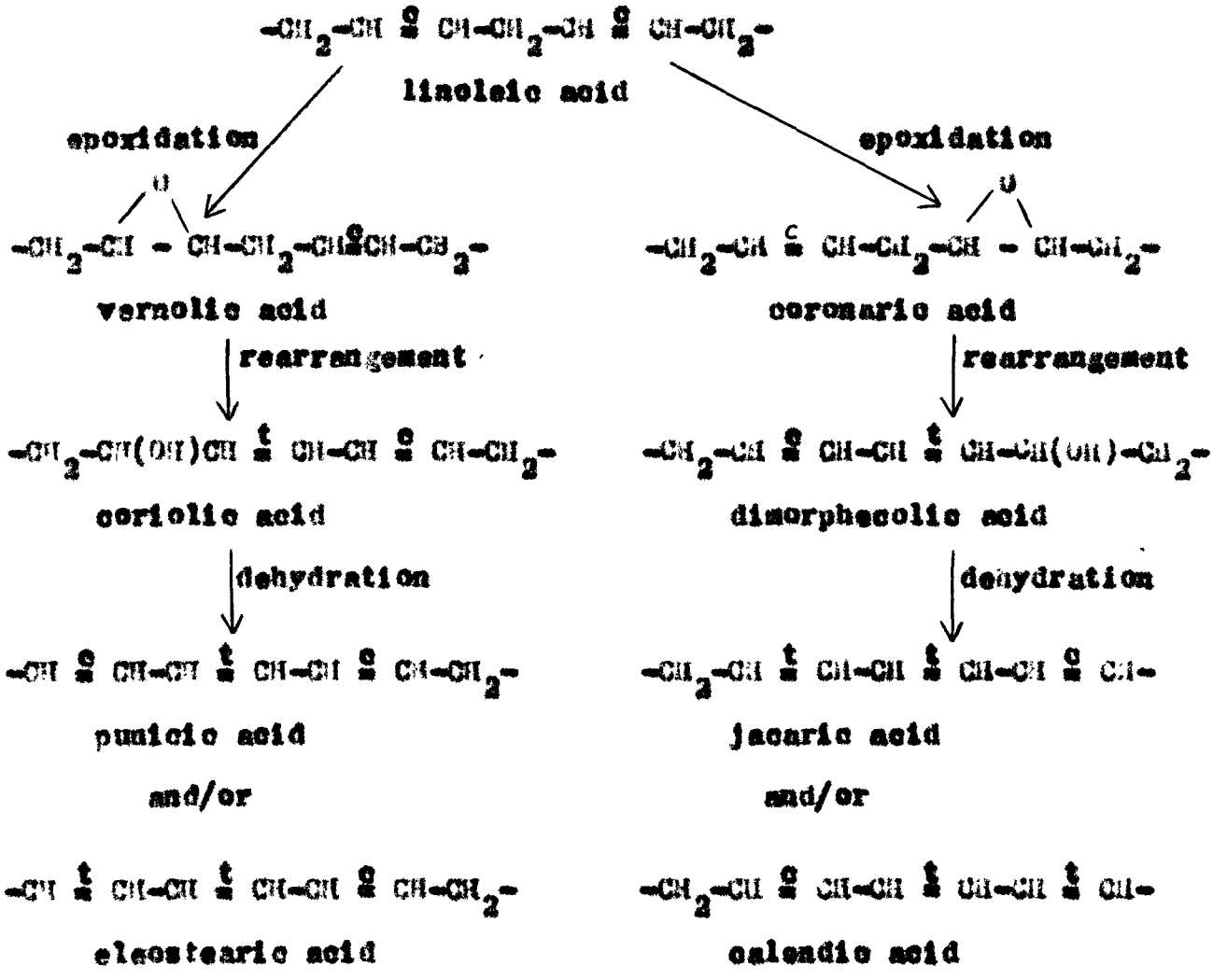
ethereal diazomethane to esterify free acids and then shaken with methanolic sodium methoxide.[132] In view of the possible importance of epoxy compounds in lipid chemistry, more sensitive methods are required for their isolation from the seed glycerides. Morris et al.[133] prepared vernolic acid from V. anthelmintica mixed fatty acids by partition between methanol and hexane. Oils having minor amount of vernolic (epoxyoleic) acid were acetolysed, saponified, and partitioned by solvents or chromatographed on thin layers of silica to isolate the resulting threo-12,13-dihydroxyoleic acid.[110,137] Isolation and identification of this acid confirmed the presence of epoxyoleic acid.[110,137] Fallent et al.[138] isolated the methylesters of epoxy acids by subjecting the mixed methyl esters to countercurrent distribution (CCD) separation. Alchornic acid was isolated by HPLC in Alchornea cordifolia seed oil.[125]

Various chemical degradative reactions[139] are run to establish the unknown structure of the epoxide in conjunction with GLC technique. The unsaturated epoxides are suitably cleaved by reaction with periodic acid (HIO_4) in dioxan and water.[139] The products are aldehydic in nature, and can be observed by GLC. When applied to methyl oleate, this reagent did not cleave the olefinic bond. Later on, Spencer et al.[140] performed destructive oxidation of epoxy acids with HIO_4 and the products were subsequently chromatographed on both polar and non-polar GLC columns, except that tetrahydrofuran was the solvent instead of dioxan.

The possibility exists that the epoxy group is a precursor of unsaturation or a product of the metabolism of unsaturated acids.

By this proposal the widely occurring linoleic acid becomes the source of the conjugated trienoic acids via known epoxy acids (vernolic and coronaric) and known hydroxy dienoic acids[141,142] as set out in Scheme 1. The epoxy fatty acids may, therefore, be of greater importance in lipid chemistry than previously imagined.

Scheme 1



Potential industrial interest in epoxidised oils from natural sources is derived largely from the demand for chemically epoxidised vegetable oils used as stabilisers for plastic materials[143] and also as starting materials for preparation of other long-chain compounds.[144] Replacement of epoxidised soybean oil with a biosynthetically produced epoxy oil would effect a considerable energy saving.

Gunstone[141] has suggested that the epoxyacyl groups may have biosynthetic significance as intermediates in some plant seeds. Not only this, these compounds are considered to not intrinsically active biologically, their photochemical and thermoxidation products may also be active, even more than their precursors. Swern et al.[145] have reported that epoxy fatty acids are carcinogenic for the subcutaneous tissue of mice.

(3) Hydroxy fatty acids

Natural long-chain hydroxy acids are conveniently divided into three categories. One group has the hydroxyl function at or near the carboxyl or methyl end of the chain whilst those with mid-chain hydroxyl groups can be subdivided into acids with or without conjugated unsaturation. The end-chain hydroxy acids are most likely to have this oxygenated function α or β with respect to the carboxyl group or $\omega 1$ or $\omega 2$ at the methyl end. α-Hydroxy acids occurring in seed oils[27,146,147] are α-hydroxy derivatives of acids such as oleic, linoleic, linolenic, or sterculic, with

which they usually co-occur. C_{18} mid-chain hydroxy acids without conjugated unsaturation appear to be hydration products of oleic, linoleic, or linolenic acid. Hydration of an unsaturated alkene can, of course, yield two hydroxy compounds but the natural process under enzymic control could well be regiospecific. These unsaturated hydroxy acids have the combined properties expected of an alkene and an alcohol. They also show reactions requiring the presence of both groups and the relative position of the two functional groups then becomes significant. Mid-chain hydroxy acids with conjugated unsaturation can be further categorised into those which contain and those which do not contain acetylenic unsaturation. Those acids with unsaturation which is entirely olefinic resemble the products of oxidation of polyene acids such as linoleic. Published information concerning hydroxy fatty acids from plants has been reviewed by Downing.[148]

The only hydroxylated vegetable oil available at present in rather large commercial quantities is castor oil, which contains 90% of ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid in its triglycerides. The industrial importance attained by this oil in numerous applications, including protective coatings, plastics, and synthetic intermediates, suggests that additional hydroxylated acids may extend the range of usefulness of this compound and find immediate usage in industry. Siddiqui and Osman,[149] from author's laboratory, reported the presence of ricinoleic acid in *Hiptage benghalensis* (Malpighiaceae) seed oil to the extent of 51%. An

isomer of ricinoleic acid, strophanthus (9-hydroxy-cis-12-octadecenoic) acid was first isolated and characterised by Gunstone[150] in seed oils of the genus Strophanthus. Ansari et al.,[151] from author's laboratory, reported the presence of this acid in seed oils of two Wrightia species viz., W. tinctoria and W. tomentosa (Apocynaceae) to the extents of 70 and 67%, respectively. Recently Siddiqui et al.,[152] from author's laboratory, found the outstanding high content (76%) of strophanthus acid in W. coccinea seed oil.

Seed oils of the genus Lesquerella resemble castor oil more than they do others of the plant family Cruciferae to which they belong. Three hydroxy acids related to ricinoleic acid were isolated from seed oils of various Lesquerella species: lesquerolic (14-hydroxy-cis-11-eicosenoic) acid in L. lasiocarpa,[153] densipolic (12-hydroxy-cis-9-cis-15-octadecadienoic) acid in L. densipila,[154] and auricolic (14-hydroxy-cis-11,cis-17-eicosadienoic) acid in L. auriculata[155] seed oils. Lesquerolic acid is a homologue of ricinoleic acid and auricolic acid a homologue of densipolic acid. Apart from the structural resemblance between these C_{18}/C_{20} pairs, the compounds usually co-exist suggesting that they are biosynthetically related. Recently Plattner et al.[156] reported the presence of lesquerolic acid in Heliophila amplexicaulis seed oil along with a trace of new fatty acid, 16-hydroxy-cis-13-docosenoic acid. Smith and Wolff[146] discovered a very unusual hydroxy triene acid, α-hydroxy linolenic (2-hydroxy-cis-9,cis-12,cis-15-

octadecatrienoic) in the seed oil of Thymus vulgaris (Labiatae). Later on Bohannon and Kleiman[147] isolated α-hydroxyoleic, α-hydroxylinoleic, and α-hydroxylinolenic acids from Salvia nilotica (Labiatae) seed oil. Recently from author's laboratory, two new non-vicinal dihydroxy acids, 9,14-dihydroxyoctadecanoic and 11,13-dihydroxy-trans-9-tetracosenoic (axillarenic) acids have been reported in Pegenum harmala (Rutaceae)[157] and Baliospermum axillare (Euphorbiaceae)[158] seed oils.

α-Kamlolenic (19-hydroxy-cis-9,trans-11,trans-13-octadecatrienoic) acid[159] was isolated from Trewia nudiflora (Euphorbiaceae) seed oil. Ligthelm[160] isolated and characterised conjugated acetylenic hydroxy acid, ximenynolic (8-hydroxy-trans-11-ene-octadec-9-ynoic) acid from the seed oil of Ximenia caffra (Olacaceae) and Riley[161] discovered isanolic (8-hydroxy-17-ene-octadec-9,11-diynoic) acid from Ongokea gore (Olacaceae) seed oil. Recently Miller et al.[162] isolated for the first time two new acetylenic hydroxy acids, 8-hydroxy-octadeca-10,12-diynoic and 8-hydroxy-17-ene-octadec-10,12-diynoic acids form the seed oil of Ongokea gore (isano). An acetylenic analogue of dimorphecolic acid, helenynolic (9-hydroxy-trans-10-ene-octadec-12-ynoic) acid was discovered by Powell et al.[163] in the seed oil of Helichrysum bracteatum (Compositae). A C_{17} hydroxy acid from the oil of Acanthosyris spinescens (Santalaceae) has been reported[164] and this new acid, 7-hydroxy-trans-10,trans-16-heptadecadiene-8-ynoic, is unique from a number of standpoints and poses some interesting biosynthetic problems.

The hydroxy fatty acids require extra precautions, as compared to the non-hydroxy fatty acids, in processing after hydrolysis of its triglycerides. One problem arises from their low solubility in hexane or petroleum ether. The second problem arises form the tendency of the hydroxyl group to react with the carboxyl group of the same or adjacent molecule, to form lactides or lactones. A third problem occurs with hydroxy acid in which there is a double bond adjacent to the hydroxyl group (in the allylic position). Such hydroxy acids readily dehydrate on heating, and acidic conditions of cleavage or esterification either produce dehydration or ether formation.

The isolation of hydroxy acids in pure form is generally carried out by adsorption column chromatography. If the free acids are used, they may be isolated with a silica gel (silicic acid) column. The methyl esters of hydroxy acids can be separated on a silica gel column using pentane containing 20% ethyl ether as solvent. Adsorption column chromatography has also been very effective for separating saturated acids from unsaturated one. This can be accomplished by converting the unsaturated hydroxy acids, as their methyl esters, to somewhat more polar compounds which are then readily separated with a Florisil or silica gel column. A suitable derivative is the methoxy mercuriacetate adduct, formed by warming the ester with mercuric acetate in methanol. After removal of the excess reagent by solvent partition, the mixture is added to the column of Florisil, the saturated hydroxy

fatty esters are eluted with hexane-ethyl ether (9:1, v/v) and the unsaturated esters with ethanol-chloroform-conc. HCl (10:9:1, v/v). The strong acid regenerates the unsaturated ester and the ethanol-chloroform elutes it from the column. The mercury adducts can also be eluted without decomposition and it is possible to achieve separation according to the number of double bonds, but the yields seem to fall off after the monoenes are eluted. The problem of separating saturated from unsaturated hydroxy acids can also be handled with silver nitrate-silica gel column. The isolation of hydroxy acids by means of TLC is comparatively simple and more effective. Thin-layer of silica gel G is used as the adsorbent and hexane-ethyl ether (85:15, v/v) is used as the developing solvent. TLC versions of the two methods described above are applied to separate the unsaturated hydroxy fatty esters.

GLC has also been used[185] for the separation of hydroxy fatty esters from non-hydroxy fatty esters using silicone SE-30, silicone QF-1, and polymeric ethyleneglycol succinate (EGS) as liquid phases. The separation is accomplished with the methyl esters after etherification of the hydroxyl group with methyl iodide and silver oxide. It has been found that poor recoveries and poorly shaped peaks were obtained if the hydroxyl group was not masked. Because of the methoxyl group, the ether esters come off the chromatograph roughly 1.5 C-numbers after the corresponding non-hydroxy fatty esters.

The OH group of hydroxy fatty esters can also be masked by acetylation. GLC separation of acetate derivatives seems distinctly inferior to that of ether derivatives, as the acetate derivatives require a higher column temperature for their emergence. However, if the hydroxy fatty esters are to be isolated from the gas chromatograph and degraded chemically, the acetate derivatives are preferable as they are much easier to hydrolyse. Trimethylsilyl ethers of hydroxy fatty esters have also been used for the GLC analysis. They are readily formed by combining with trimethylchlorosilane and hexamethyldisilazane. The trimethylsilyl ethers are generally eluted much faster than the acetate esters.

The position of the OH group in the carbon-chain can be fixed by GLC analysis, provided a suitable collection of authentic samples are available. The various chemical degradative methods are best suitable for hydroxyl location but the presence of double bonds in hydroxy acids make degradative studies more difficult. Recently mass spectrometry is the method of choice for the structural elucidation of hydroxy acids.

DISCUSSION

For many centuries, various fats and oils have served as sources of lubricants, cosmetics and soaps; but their component fatty acids have been recognised and used only since the work of Chevreul, who made outstanding contributions to understand the structure of fats in the period 1813-1825. Since that time, fatty acids have become essential to man in a multitude of uses. A survey of literature on seed oil analysis revealed that a large number of uncultivated plant species yield seeds whose oils are of unusual composition as compared to the conventional seed oils. During recent years it has been emphasised by Indian Oil Technologists to start a drive for exploring the wild oilseed potential of our country. More emphasis on the production and utilisation of minor oilseeds that are not presently in cultivation, may substantially ease problem of shortage in oils now being faced by the country. With this view a programme was been underway at the author's laboratory to determine by chemical screening the general characteristics of seed oils from a large number and variety of presently uncultivated oil-exuding seeds.

In an attempt to search for the new oilseed crops for industrial as well as edible purposes, seed oils[166] of a wide array of wild species of different plant families were analysed for their component fatty acids. The major features are: (i) isolation and characterisation of 73.5% (highest levels) of tripalmitin in Ochna squarrosa (Ochnaceae)[167] glycerides;

(ii) isolation and characterisation of hydroxy fatty acids in Hiptage benghalensis (Malpighiaceae),[149] Wrightia tinctoria, W. tomentosa[151] and W. coccinia[152] (Apocynaceae), Pegamum harmala (Rutaceae),[157] Balliospermum axillare (Euphorpiaceae),[158] and Plantago major (Plantaginaceae)[163] seed oils; (iii) characterisation of cyclopropenoid fatty acids in Althaea officinalis,[169] Sida acuta, S. rhombifolia,[170] S. grewioides, Hibiscus cannius,[171] H. subdariffa[172] and Urena lobata[173] (Malvaceae), and Pentapetes phoenicea[169] and Kariolaena hookeriana[103] (Sterculiaceae) seed oils; (iv) isolation and characterisation of epoxy fatty acids in Vernonia roxburghii (Compositae),[174] and Mucuna pruriens (Leguminosae)[173] seed oils; (v) isolation and characterisation of isoballenic acid in Leucas cephalotes[76] and L. urticifolia[77] (Labiatae) seed oils; and (vi) isolation and characterisation of cyanolipids in Cardiospermum canescens[175] and Dodonea viscosa[176] (Sapindaceae), and Heliotropium indicum and H. eichwaldi[177] (Boraginaceae) seed oils.

In continuation of this programme, the work described in Part-I of this thesis deals with the oil characteristics, seed properties, and fatty acid composition of herbaceous oilseeds coupled with the isolation and characterisation of unusual fatty acids from them.

Analysis of Herbaceous Seed Oils

As a part of extensive programme of research aimed at discovering new seed oils and fatty acids which may lend themselves to practical utilisation, a number of seed samples[166] from plants were flowed in for oil analysis at author's laboratory. In the present study, seeds of wild species representing eight botanical families which include nine species, being reported for the first time and one species already reported earlier,[173] were evaluated for their oil content, physico-chemical characteristics, and fatty acid composition. Seed oils obtained in yields below 5% were examined with the expectation that a fatty acid of novel structure which would be of academic interest and might have practical value also, might be discovered.

The air-dried seeds were powdered and extracted thoroughly with petrol in a Soxhlet. Moisture, oil, and protein on powdered seeds as well as physico-chemical characteristics of oils were determined according to the official and tentative methods recommended by AOCS.[1] The results are depicted in Table I. Oil content of wild species showed a wide range of variation from 0.9% in Lathyrus aphaca to 28.6% in Sesamum indicum. The species Ipomoea carnea was found to contain similar amounts of oil and protein as reported by Barclay and Earle[179a] in the screening of this species. The estimated protein content of all defatted seeds on a dry basis, 9.5-32.0% is lower than that of usual oilseed meals, but adequate to be useful as a feed material.

The analytical data reveal that the breeding programme could develop plants suitable for mechanical production and harvesting, and possible also could vary the oil as well as protein composition to increase its value for specific applications.

The oils were examined by various chromatographic and spectroscopic techniques to evaluate their fatty acid compositions. The IR spectra of the oils had maxima at 3000 (s) and 1650 (w) cm^{-1}, indicating ordinary unsaturation. There were no peaks in the region of trans and conjugated unsaturation, and for any unusual functional group. Similarly, the UV spectra of the oils showed no evidence of conjugated double bonds. The picric acid TLC test[26] for epoxy function and Halphen test[27,28] for cyclopropenoid material in the oils were found negative for all the species. The chromatographic techniques did not reveal the indications of unusual component fatty acids or interferences with the applications of standard methods. Argentation TLC[29a] of the methyl esters gave clear spots corresponding to the saturates, monoene, and diene in Agenthospermum hispidum and Gebaokia chinensis parallel to those from authentic linseed esters resolved alongside. The methyl esters in remaining eight species indicated the presence of the spot for triene also in addition to the spots for saturates, monoene, and diene on silver-ion TLC. Reversed-phase TLC[19] of the esters confirmed the presence of C_{16} and C_{18} saturated acids in all the samples, and also C_{12} saturated acid in Polygonum serrulatum, P. indicum, and P. chinensis.

Fatty acid composition of the oils were determined by GLC. Quantitative estimation of fatty acid components on a gas chromatogram was achieved by comparing retention times with those of lipid standards (obtained from SIGMA Chemical Company, U.S.A.). The percentage of each fatty acid obtained by calculation of area under peaks on the gas chromatogram is listed in Table I. Occasionally, saturated, mono-, and polyethenoid esters were separated by preparative silver-ion TLC and re-examined by GLC. Fair agreement between the iodine value (I.V.) derived by chemical means (Wijs) and that calculated from the fatty acid composition indicate that the amounts of unsaturated acids given are substantially correct.

Perusal of the fatty acid compositional data in Table I leads to a number of interesting generalisations.

Saturated acids

As is evident from Table I, the amount of total saturated acids present in the seed oils analysed ranges from 13.6% in S. indicum to 29.5% in Rhynchosia aurea. Stearic acid, which is usually found as a minor component of the seed glycerides, is present in the range 1.5-6.9%. It is present to the extent of 6.9% in I. carnea. This species belongs to the family Convolvulaceae, in which stearic acid is nearly always present to the extent of over 10% of the mixed fatty acids of the glycerides but here and there exceptions also be noticed. The major saturated acid component

of seed glycerides viz., palmitic acid forms over 20% of the mixed fatty acids in four species; 24.7% (*R. aurea*), 24.1% (*L. aphaea*), 21.3% (*I. carnea*), and 20.3% (*O. chinensis*). In addition to C_{16} and C_{18} acids, the species *P. serrulatum*, *S. indicum*, and *O. chinensis* show the presence of lauric acid in the amount of 1.3, 0.9, and 4.0%, respectively.

Mono- and polyethenoid acids

All the seed oils exhibit good sources of unsaturated fatty acids forming over 70% of the mixed fatty acids of the glycerides. Particular attraction from a utilisation standpoint is the occurrence of oleic acid in sufficiently high concentration (42.3%) in *S. indicum* seed oil. Linoleic is the principal component acid (35.9-74.3%) amongst the unsaturated acids in all species except *Sapium insigne* and *S. indicum*. Oleic acid (42.3%) is predominant unsaturated acid in *S. indicum*, whereas linolenic acid (31.7%) is major in *S. insigne* like other Euphorbiaceae seed oils.[166] Six species are unusual in having major amount (>70%) of oleic-linoleic acids, which range up to 70.0% in *I. carnea*, 73.3% in *P. serrulatum*, 94.1% in *S. indicum*, 83.1% in *A. hispidum*, 73.7% in *Triumfetta rhomboidea*, and 74.1% in *O. chinensis*. The combined content of linoleic-linolenic acids are found major in *L. aphaea* (63.4%) and *S. insigne* (59.4%).

Chemotaxonomic relationships

A comparison of the results of the present study with earlier observations lead to certain comments regarding the compositional data. Three species of Sapium have been analysed by Kleiman et al.[180] These authors reported the fatty acid distribution as 18:3 > 18:2 > 18:1 and 16:0 > 18:0. The presence of an unusual component (2,4-decadienoic) acid was reported in the seed fat of S. sebiferum which was further confirmed by Hopkins and Chisholm[181] and Barclay and Earle.[179b] The seed oil of S. insigne reported here gives no indication of any unusual fatty acid, although the pattern of distribution of fatty acids is exactly same as reported.[180] Fatty acid composition of few species of Ipomoea have been reported previously from author's laboratory.[166h,p] The determination of polyethenoid acids in I. carnea confirms previous reports on the pattern of composition of Ipomoea (18:2 > 18:1 > 18:3) but no traces of C_{16} and C_{20} acids are observed. Recently Kapoor et al.[132] determined the properties and fatty acid composition of two varieties of Sesbania sesban. These authors reported the fatty acid composition as: 18:3 (6.5 and 6.7%), 18:2 (42.0 and 43.6%), 18:1 (34.2 and 33.3%), 16:1 (1.3 and 1.9%), 16:0 (12.2 and 12.9%), and also 20:0 (3.1 and 1.5%). Oil of Sesbania aegyptica in the present study shows same pattern of fatty acid composition but has less amount of 18:1 acid and no trace of C_{20} acid as compared to S. sesban, while it shows the presence of 18:0 acid in addition. This difference in fatty acid

composition may be a reflection of the effects of the soil and climate, and also may result from differences in the source of samples.

Report about the fatty acid composition of one seed oil analysed in the present study is available from literature. Jamieson and Baughman[183] have determined the fatty acid composition of S. indicum by conventional methods. The compositional data of S. indicum obtained here by GLC are found more or less similar to that reported previously.

'Drying', 'semidrying', and 'nondrying' oils

Seed oils rich in mono- and polyethenoid fatty acids can serve as intermediate raw materials for industrial utilisation as 'drying', 'semidrying', and/or 'nondrying' oils depending upon the relative proportions of oleic, linoleic, and linolenic acids. The polyethenoid-rich seed oils, especially those in which content of linoleic and/or linolenic acid exceeds 67% are important 'drying' oils based on their ability to dry or harden when exposed to air. Oils of A. hispidum and Q. chinensis approach this composition and thus belong to 'linoleic-rich drying' oils. L. aphaca and S. insigne seed oils contain moderate proportions of both linoleic (35.9 and 27.7%) and linolenic (27.5 and 31.7%) acids like candlenut and rubberseed oils and may be of value as 'drying' oils. Four seed oils, S. aegyptica, P. serrulatum, S. indicum, and P. rhomboidea, containing linoleic acid 40-60.

are 'semidrying' oils like cottonseed and maize oils. H. aurea and I. cornea oils have linoleic acid 36.3 and 35.6%, respectively, and hence can be classified as 'nondrying' oils.

In summation it may be added that the present screening data may lead to explore some of the plant species for obtaining new oilseeds with more desirable pattern of fatty acid composition. Further, the species rich in oils as well as in specific acid could be of great strategic importance and, if sufficiently economic, could prove of value to the chemical industry in normal times. The fatty acid composition of T. rhomboidea in the present study is found near about similar to that of soybean oil.

EXPERIMENTAL PROCEDURES

Materials and Methods

(i) Source of oilseeds

The seed samples were collected from the uncultivated areas and were well identified by the staff botanists. Few of them were purchased from a commercial seed house. They were of the current year's stock.

(ii) Extraction of Oil

All seed samples were freed from seed fragments and dockage and then grounded in a disintegrator. The powdered seeds were extracted exhaustively with petroleum ether (40-60°C) in a

Soxhlet apparatus and the extracted oil was neutralised by passing it (~1g) in chloroform solution through a short column of alumina (10 g). The analytical values of oils and seeds were determined according to the procedures recommended by the AOCS methods[1] and the data are summarised in Table I.

(iii) Preparation of mixed fatty acids

Seed oil was refluxed with ethanolic potassium hydroxide. The unsaponifiable material was removed and the free fatty acids were obtained in the usual manner. Wherever necessary, saponification was carried out in cold condition under nitrogen.

(iv) Preparation of methyl esters

Esterification was carried out as follows, except where specified. Samples were refluxed for 1 hr in a large excess of anhydrous methanol containing 1% sulphuric acid (v/v). In each case, resulting mixtures were diluted to the cold point with water, chilled in an ice bath, and then extracted repeatedly with ethyl ether. Combined extracts were dried over anhydrous sodium sulphate and evaporated in vacuo. In special cases methyl esters were prepared by methanolysis with sodium methoxide (0.4%) or by treatment with ethereal diazomethane. Yields of recovered esters ranged from 96 to 97%.

(v) Thin-layer chromatography (TLC)

Analytical TLC was performed on plates covered with 0.25 mm or 1.0 mm layers of silica gel G or 20% silver nitrate-impregnated silica gel G. Binary mixtures of ethyl ether and petroleum ether were used as developing solvents. The plates were rendered visual by spraying with a 20% aqueous solution of perchloric acid and heating in an oven ($\sim 110^{\circ}C$) for 10 min. Preparative plates were sprayed with a 0.2% ethanolic solution of 2',7'-dichlorofluorescein and then viewed under ultraviolet light. Separated components were recovered in 85-95% yield from preparative plates by slurrying the adsorbent with ethyl ether. For reversed-phase TLC, the coated plates were uniformly impregnated with silicone oil. Solvent system acetonitrile-acetic acid-water (70:10:20, v/v) was used for development.

(vi) Gas-liquid chromatography (GLC)

GLC analysis for the quantitative examination of metayl esters was carried out with Perkin-Elmer Model 154 equipped with a thermal conductivity detector using stainless steel packed column (2 ft x 1/8 in) coated with diethylene glycol succinate (DEGS, 15% on chromosorb W, 45-60 mesh) and a (2 ft x 3/16 in) column of silicone (SE 30, 2%). The separations were carried out isothermally at $200^{\circ}C$. Hydrogen at a flow rate of 70 ml/min was the carrier gas and chart speed was 30 in/hr. Quantitation was done by calculation of area under peaks on the gas chromatogram

with the help of integration and results are quoted as composition (% wt). The percentage of each ester was calculated as the percent of the total area of all the peaks.

(vii) Infrared (IR) spectroscopy

IR spectra were determined on a Perkin-Elmer 621 spectrophotometer as liquid film or as 1% solutions in carbon tetra chloride (CCl_4) or carbon disulphide (CS_2).

(viii) Ultraviolet (UV) spectroscopy

UV measurements were made in methanolic solutions with a Beckmann DK-2A spectrophotometer.

Fatty Acid Spectrum of Cruciferae Seed Oils

In the last fifteen years, there has been much interest in obtaining new industrially usable oilseeds, both for edible and for purely technical purposes. Special attention has been paid to Cruciferae as this family, contrary to most other oil crops, combines good harvesting yields with good growth capability in temperature climate. Several comprehensive investigations exist within this field,[62,63,134-136] but even so about 10% of the nearly 3000 species of this family have been studied to date.

Several species of the family Cruciferae produce seed oils which differ in fatty acid composition from other vegetable oils. They are noted for the presence of C_{20}, C_{22}, and C_{24} acids, particularly the monoene members, erucic (22:1) and eicosenoic (20:1) acids. The majority of them contain 25-50% erucic acid. However, some are richer in 20:1 than 22:1, and a few contain virtually no C_{20}, C_{22}, or C_{24} acids and are instead rich in α-linolenic acid.[62,63,183,186]

While the majority of the Cruciferous oilseeds are used in edible products such as edible oils, margarine, and shortening, some are utilised as raw materials for various technological purposes. The oils obtained from Cruciferous seeds, although of greater value in margarine manufacture, suffer from a fatty acid composition which limits their usefulness in certain edible oil products. The high erucic acid content gives the oil a high

melting range. Furthermore low linoleic acid content excludes the use of such oils as a major constituent of brands of margarines, which, for nutritional reasons, contain a certain minimum percentage of that acid. The rather high linolenic acid content, on the other hand, yields a reduced flavour stability of rapeseed-oil based products.

Furthermore it seemed to be of interest to the plant breeder as well as to the vegetable oil processor to know the over-all variability in fatty acid composition of seeds of these varieties.

Considering the above facts, much interest has been shown in recent years in finding seed sources free from erucic and linolenic acids and high in linoleic acid, and rich in erucic acid in wild and uncultivated species of Cruciferae. With this aim a study of Cruciferous seed oils is initiated in order to search for 'new' oilseed crops having favourable lipid composition, viz., zero or low erucic and linolenic acids and high linoleic acid for the food industry, and high erucic or high linolenic acid for industrial raw material. In the present study, seeds of the cultivated species Iberis (Candytuft) I. amara and I. odourata, and Cheiranthus cheiri (Wallflower), of Cruciferae were analysed for their oil content and fatty acid composition.

The analytical data on the seed samples and derived oils were determined according to the procedures recommended by A.O.C.S.[1] and

are depicted in Table II. The seeds on extraction with petroleum ether yielded oil 26.0% in I. amara, 36.2% in I. odourata, and 39.2% in C. cheiri, while the remaining cake contained about 30% protein in all three species. The oils were clear fluid with a faint yellow colour like mustard oil. Turbidity,[187] picric acid,[26] and Halphen[27,33] tests showed the absence of hydroxy, epoxy, and cyclopropenoid fatty acids in all the seed oils. UV and IR spectra of the oils showed no conjugation/trans unsaturation or any unusual functional group. Direct TLC of each of the oils and methyl esters confirmed this observation. There was no evidence for the hydroxy fatty acid to be present in any sample as reported in some Cruciferous seed oils.[153-156]

Argentation TLC[9a] of the methyl esters gave clear spots corresponding to the saturates, monoene, diene, and triene in all the samples parallel to those from authentic mustard esters resolved alongside. Unusual observation on thin layers of silica impregnated with silver nitrate was noted for C_{20} and C_{22} monoenes. The spot for monoene higher than 18 carbon atoms appeared between the spots for saturates and for C_{18} monoene. The R_f value of this spot for higher monoene was identical to that of mustard esters used as reference standard. Reversed-phase[10] TLC of the esters confirmed the presence of C_{18}, C_{18}, and C_{20} saturated acids in all the three esters. I. amara and C. cheiri were also found to contain C_{22} saturated acid.

The prevalent fatty acids have been identified only by chromatographic characteristics. Data from the two columns (SE 30, 2% and DEGS, 15%) were used to calculate the weight percentages of the component acids. Identification of the component acid was made by comparing its retention time with that of the reference lipid standard (SIGMA) and the percentage of each fatty acid obtained by calculation of area under the peaks in the gas chromatogram is listed in Table II.

The fatty acid composition of seed samples reveals that these three seed oils contain monounsaturated acids with 20 and/or 22 carbon atoms in amounts typical of many Cruciferae, and that the amounts of other acids are similar to those commonly found in seed oils. Within the genus Iberis, the fatty acid composition (Figure 1) varies considerably as seen by comparing the two species investigated here. Oil from I. odourata is rich in eicosenoic acid (41.9%) and contains no erucic acid. On the other hand I. amara seed oil contains eicosenoic acid as minor component (5.3%) and erucic acid as major component (43.4%). Here it appears that large proportion of C_{22} monoene in I. odourata does not necessarily preclude the formation of erucic acid in the species. However, the general composition of conventional acids is usual in both instances. The oil of C. gheiri contains both eicosenoic (11.5%) and erucic (22.9%) acids along with eicosadienoic acid (2.3%) as minor constituent. By comparing the fatty acid composition of two species, it is observed that decrease in the

TABLE II

Analytical Data on Cruciferae Seeds and Oils

Property	Value		
	Iberis amara	Iberis odourata	Cheiranthus cheiri
Composition of seeds (%)			
Moisture	5.2	7.5	4.7
Oil	26.0	26.2	29.2
Protein (N x 6.25)	30.1	31.9	28.0
Oil characteristics			
Refractive index, n_D^{30}	1.4800	1.4655	1.4710
Iodine value (Wijs)	110.8	106.7	133.6
Saponification value	170.2	170.5	178.0
Unsaponifiable matter (%)	2.0	1.8	1.3
Fatty acid composition (area, %)			
16:0	3.5	3.9	6.5
18:0	1.4	1.9	1.1
18:1	26.0	36.2	12.9
18:2	12.3	7.9	17.8
18:3	9.1	8.2	16.1
20:1	5.3	41.9	11.5
20:2	-	-	2.3
22:1	43.4	-	29.9

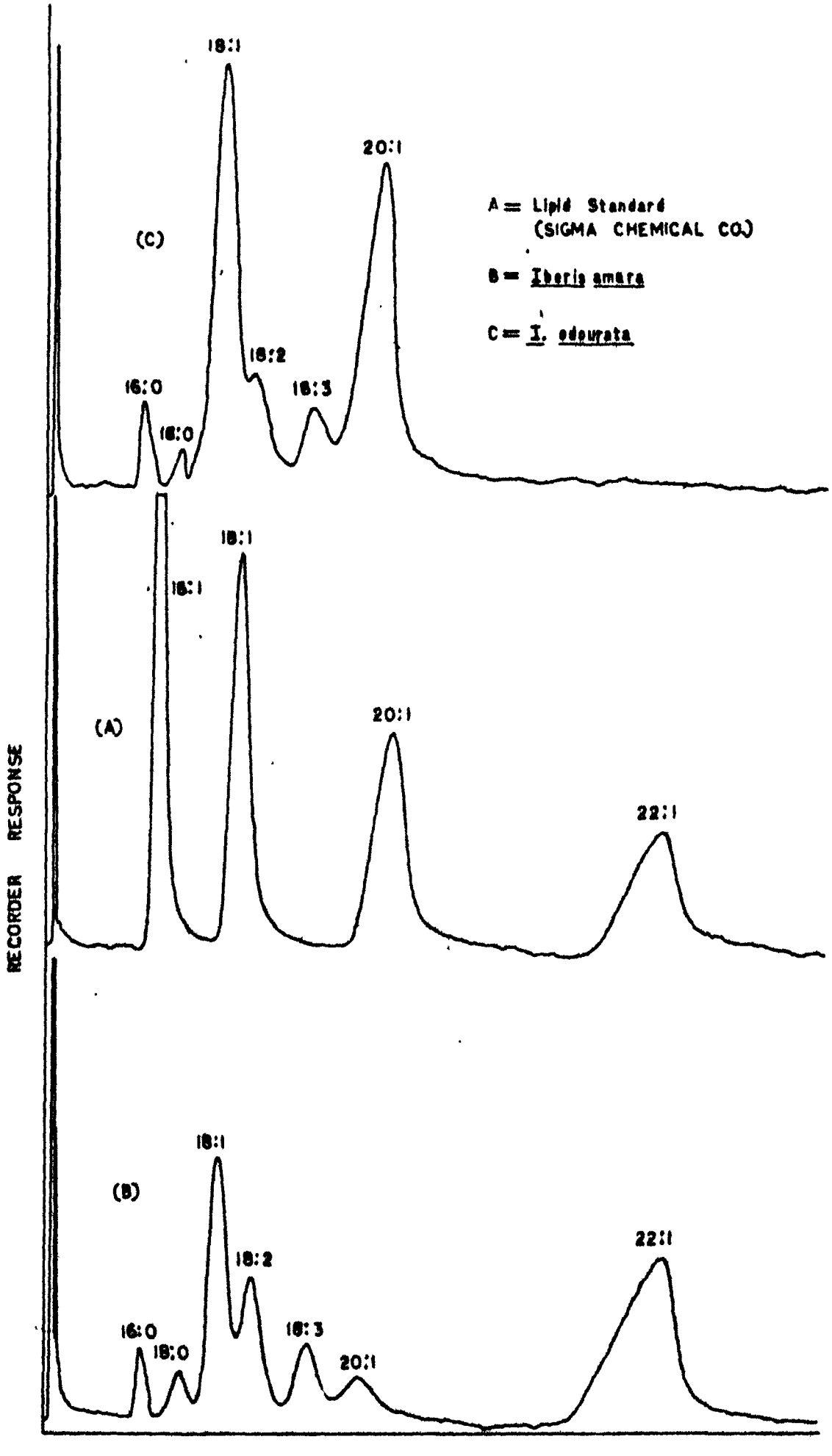


Fig.1. GAS CHROMATOGRAMS OF METHYL ESTERS

concentration of erucic acid in C. cheiri results in an increase of C_{16} and C_{18} acids, more particularly in linolenic acid, as compared to I. amara.

The properties and fatty acid composition of two species screened are reported in literature. Nikolajczak et al.[134] have analysed the American variety of I. amara for its oil and component acids. The species shows almost similar oil content while its protein content is slightly high (30.1 vs 28.0%) with respect to the previous report on the species. The American variety was reported to have 16:0 (3%), 16:1 (0.3%), 18:0 (0.6%), 18:1 (19%), 18:2 (18%), 18:3 (12%), 20:1 (6%), 20:2 (0.3%), 22:1 (38%) and 24:1 (2%) in contrast to 16:0 (3.3%), 18:0 (1.4%), 18:1 (36.0%), 18:2 (12.3%), 18:3 (8.1%), 20:1 (5.3%) and 22:1 (43.4%) found in the present sample. Nikolajczak et al.[134] and Jart[136] have reported the oil content and fatty acid composition of American and German varieties of C. cheiri. The species is found to possess oil content same to that of American variety and comparatively high (29.2 vs 23%) with respect to German variety. The fatty acid composition of C. cheiri found in the present study is somewhat similar to the composition reported earlier by these authors. The difference is the absence of 16:1, 20:0, 22:0, 22:2, 24:0, and 24:1 acids, as minor constituents, in the present sample. The discrepancy in composition may be due to the environmental factors.

The species I. odourata has not been studied before. Although two other species, I. umbellata[134] and I. pruitii[63] have been reported in literature for their component acids, but none of them has been found to be of 'zero erucic' group like I. odourata found in the present study. Both species of the genus Iberis, from which seeds were collected for the present study, were grown in same place, under same conditions, and at same time. Therefore, it seems that the marked difference in fatty acid composition between species of the same genus is due to genetic factors and not due to environmental factors.

In conclusion it may be added that the presence of erucic acid in I. amara and absence of this characteristic Cruciferae seed oil acid in I. odourata seed oil is interesting from chemo-taxonomic point of view as both the species belong to the same genus. The discovery of a unique oil during examination of this small sampling of the family Cruciferae encourages further exploration of the plant world for new chemical composition. Dissimilarity of the two species of Iberis demonstrates the need for examination of all available species rather than for a cursory sampling of families and genera.

The sufficient oil content and resemblance of fatty acid composition of I. amara seed oil with that of mustard oil warrant further studies to appraise its crop potential and to assess its practical value for providing 'new' edible oilseed. None of the species analysed was found to be linoleic or linolenic rich oilseed.

I. odourata contains no erucic acid and instead rich in eicosenoic acid. The adverse effects of feeding diets containing rapeseed oils with high levels of erucic include a depression in growth,[188] an early accumulation of cardiac lipid,[189] as well as a high incidence of myocardial necrosis.[190] Considering these effects, I. odourata oil with zero erucic may be suitable for food industry. As the oil is rich in eicosenoic acid, it may also be used in synthesising wax ester intermediate for prospective lubricant additives and PVC plasticisers. The present study reveals that both the species of Iberis (Candytuft) and the species C. cheiri (Wallflower) are not only the ornamental sources of gardens, but they also possess industrial and edible importance.

EXPERIMENTAL PROCEDURES

Materials and Methods

Source of oilseeds

All the three seed samples were collected from National Botanical Research Institute (N.B.R.I.), Lucknow (India).

The experimental procedures, extraction of oil, preparation of mixed fatty acids and methyl esters, TLC, GLC, UV and IR spectroscopy, were all same as described in the previous section Analysis of Herbaceous Seed Oils.

Note: After completion of our work, Badami and Patil[200] reported the fatty acid composition of I. odourata seed oil using reversed phase TLC but no GLC. The results are however different from our findings.

HBr-reactive Fatty Acids in *Abutilon indicum* (Malvaceae) Seed Oil

It is well known that the biogenetic oddity associated with Malvaceous seed oils is that seed oils containing cyclopropenoid fatty acids usually contain measurable amounts of epoxy fatty acids.[137] In anticipation of the co-occurrence of cyclopropenoid and epoxy acids in the seed oils of Malvaceae, it was considered worthwhile to re-examine the seed oil of *Abutilon indicum*.

A. indicum (Var. Populifolium; Hindi, Kanghi) of Malvaceae is abundant throughout the hotter part of India. Seeds, called as Kalbij, are dark brown minutely stellate-hairy. The seed fat of *A. indicum* has been examined previously by several workers.[191,192] These authors have not shown the presence of HBr-reactive acids in its oil. Later on, in the screening of seed oils it was shown that HBr-reactive acids occur in the glycerides of seed oils of the genus *Abutilon* (Malvaceae).[137,179c,193] In continuation of earlier work on HBr-reactive acids containing seed oils reported from this laboratory,[172,173] examination of *A. indicum* seed oil has revealed significant amount of unusual constituents.

Durbetaki titration[30a] of freshly extracted oil of *A. indicum* at two different temperatures[194] indicated the presence of approximately 5% HBr-reactive acids. The oil gave positive Halphen test,[33] thereby indicating the presence of cyclopropenoid material. Analysis of oil by TLC revealed three spots (R_f = 0.51, 0.44, and 0.36). The spot at R_f 0.44 gave positive picric acid TLC[36] test, indicating

the presence of epoxy acid in the seed glycerides. The oil gave no indication of conjugation in its UV measurement and had the characteristic IR band at 1010 cm^{-1} for the cyclopropene moiety, and a weak band at 948-926 cm^{-1} attributed to epoxy group.

Because of the reactivity of the epoxy acid, it was converted into the corresponding dihydroxy acid without destruction of the cyclopropene ring by treatment with acetic acid-10% sulphuric acid (5:2, v/v) at room temperature, following the procedure of Wilson et al.[110] Oil recovered from the acetic acid-sulphuric acid treatment was saponified by stirring overnight with 0.5N alcoholic KOH at room temperature. The liberated fatty acids were separated into oxygenated and non-oxygenated fractions by preparative TLC and undertaken separately for the characterisation of individual fatty acids.

Characterisation of non-oxygenated fatty acids

The concentrate of non-oxygenated fraction (hydrogen bromide equivalent, HBE = 3.43; Halphen test positive) was esterified with ethereal diazomethane. The methyl esters showed the characteristic IR band at 1010 cm^{-1} (cyclopropene). There was no absorption at 3500-3400 cm^{-1} (hydroxyl), 948-926 cm^{-1} (epoxy) or 3320 and 2120 cm^{-1} (acetylenic).

Methyl esters was analysed by GLC after treatment with saturated solution of silver nitrate in absolute methanol to form stable derivatives of cyclopropenoid acids, following the procedure

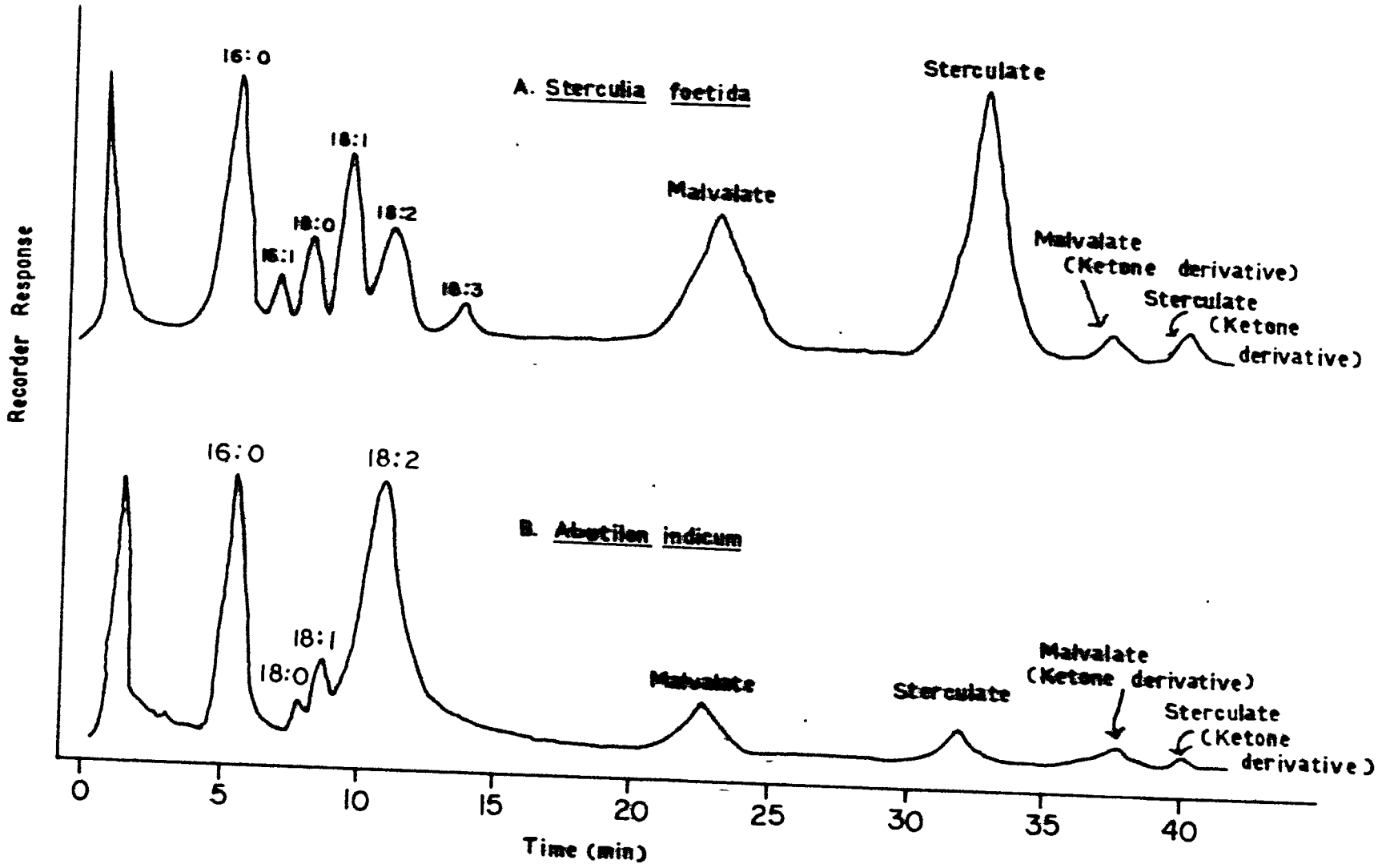


Fig. 2. GLC of $AgNO_3$- MeOH Treated Methyl Esters

of Schneider et al.[118] The GLC analysis (Figure 2) clearly established the presence of malvalic and sterculic acids in this fraction by a comparison of the relative retention times of the derivatives of Sterculia foetida esters used as reference standard. Acids other than cyclopropenoid were identified as palmitic, stearic oleic, and linoleic, which were further confirmed by reversed-phase and argentation TLC techniques. Direct TLC showed only non-oxygenated acids. The reversed-phase TLC revealed a spot near the starting point corresponding to the spot exhibited by S. foetida esters. Clear spots for usual critical pairs were also obtained. Argentation TLC showed spots for saturates, monoene, and diene parallel to those obtained from S. foetida esters resolved alongside. GLC compositional data (Table IV) showed that this fraction contained 3.2% of total cyclopropenoid acids which is in agreement with the value obtained by Hbr-titration.

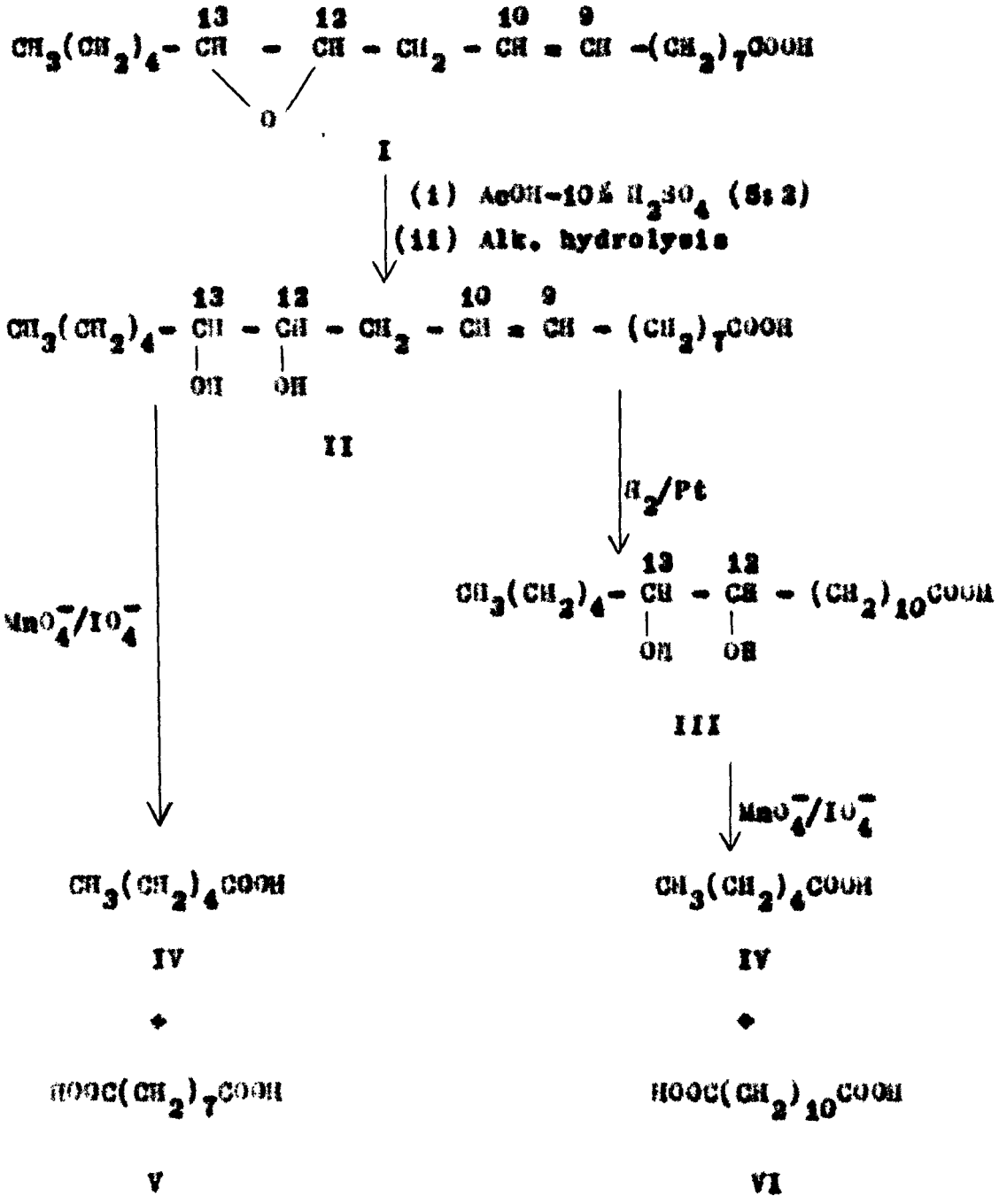
Characterisation of oxygenated fatty acid

The oxygenated fatty acid concentrate (0.48 g from 30 g of oil, equivalent to 1.6% of the weight of the oil) had IR band at 3460 cm^{-1} (broad) indicative of hydroxyl and gave no evidence of trans unsaturation (965 cm^{-1}). Quantitative determination of the oxirane content of the oil is in fair agreement with the amount of dihydroxyoleic acid isolated. The oil showed 0.08% oxirane oxygen, equivalent to 1.6% epoxyoleic acid.

Successive crystallisations of the crude dihydroxy acid from acetone and petroleum ether-ethyl ether (3:1, v/v) afforded a crystalline product, m.p. 53.5-54°C. No depression of the melting point was observed on admixture with an authentic sample of threo-12,13-dihydroxyoleic acid prepared from Vernonia anthelmintica seed oil. Co-chromatography on TLC revealed a single spot.

The dihydroxy acid (II) was characterised chemically (Scheme 2). On hydrogenation, II consumed 0.97 mole equivalent of hydrogen to yield dihydroxystearic acid (III), m.p. 96-97°C (from ethyl acetate). An admixture with authentic threo-12,13-dihydroxystearic acid had m.p. of 96-96.5°C. The absorption of 0.97 mole equivalent of hydrogen indicates the presence of one double bond in the original acid. Cleavage of unsaturated diol (II) by permanganate-periodate gave hexanoic (IV) and azelaic (V) acids. Hence the points of cleavages were at C-9, C-10, C-12, and C-13. The oxidative fission of saturated diol (III) gave hexanoic (IV) and dodecanedioic (VI) acids, showing the hydroxyls at C-12 and C-13 positions. The double bond in the unsaturated diol (II) must be between C-9 and C-10 since this was the point of cleavage when oxidised. Its IR spectrum gave no evidence of trans olefinic acid, hence the double bond has the cis configuration. Comparison of the mobility of saturated and unsaturated diols with known threo-dihydroxy acids by TLC on boric acid-impregnated silica demonstrated that both had the threo configuration. These results led to the conclusion that the dihydroxy acid derived from the oil

Scheme 2


CH3(CH2)4-CH(13)-CH(12)-CH2-CH(10)=CH(9)-(CH2)7COOH
O
I
(i) AcOH-10% H2SO4 (5:2)
(ii) Alk. hydrolysis
CH3(CH2)4-CH(13)(OH)-CH(12)(OH)-CH2-CH(10)=CH(9)-(CH2)7COOH
II
H2/Pt
MnO4-/IO4-
CH3(CH2)4-CH(13)(OH)-CH(12)(OH)-(CH2)10COOH
III
MnO4-/IO4-
CH3(CH2)4COOH
IV
CH3(CH2)4COOH
IV
HOOC(CH2)7COOH
V
HOOC(CH2)10COOH
VI

is threo-12,13-dihydroxy-cis-9-octadecenoic acid. The original component of the oil is therefore cis-12,13-epoxy-cis-9-octadecenoic (vernolic) acid.

The present work has demonstrated that in addition to the conventional acids occurring in A. indicum seed oil, the 12,13-epoxyoleic (vernolic), sterculic, and malvalic acids are present as minor constituents of triglycerides. The silver nitrate-methanol method[115] of GLC analysis for cyclopropenoid acids and the method involving acetylation of oil[110] at room temperature for characterisation of epoxy acid appear to be convenient procedures for the analysis of oils in which these acids co-occur as minor constituents in addition to normal fatty acids. The species A. indicum analysed in the present study does not show the presence of linolenic acid in its oil like other HNr-reactive fatty acids containing Malvaceae seed oils[169-173] reported from this laboratory. The absence of linolenic acid in these Malvaceous seed oils is quite unusual as the precursors of cyclopropenoid (sterculic and malvalic) and epoxyoleic acids are oleic and linoleic acids, respectively.

In conclusion it may be added that the presence of minor proportions of three biologically active fatty acids in A. indicum seed oil might adversely affect its stability and nutritional properties and deserves further research in the direction of lipid metabolism.

EXPERIMENTAL PROCEDURES

General methods

Spectroscopic and chromatographic analyses of the oil and its esters were done essentially according to the methods described in the section Analysis of Herbaceous Seed Oils. The triglycerides containing epoxy group was revealed by on-the-plate test with picric acid, as described by Fioriti and Sims.[26] The dihydroxy acid methyl ester was analysed on silica gel impregnated with boric acid and developed with hexane-ether-acetic acid (60:40:1, v/v). The spots were visualised by charring the plates at 120°C after they had been sprayed with a 20% aqueous solution of perchloric acid.

Melting points were observed on a Kofler apparatus and are uncorrected.

Preliminary analysis of the seeds and its oil by the standard AOCS procedures[1] gave the characteristics shown in Table III.

TABLE III

Analytical Data on *Abutilon indicum* Seeds and Oil

Oil content of seeds, %	10.0
Unsaponifiable content, %	2.0
Protein content, N x 6.25, %	21.3
Moisture, %	5.3
Iodine value (Wijs)	109.0
Saponification value	153.5
Refractive index, n_D^{40}	1.4764
Halphen test	Positive
Oxirane oxygen, %	0.08
HBr equiv. 3°C	1.56[a]
55°C	3.42[b]

[a] Expressed as % epoxyoleic
[b] Expressed as % cyclopropenoid

TABLE IV

Fatty Acid Profile of *Abutilon indicum* Seed Oil

Fatty Acid	wt., %
Palmitic	28.8
Stearic	4.9
Oleic	12.9
Linoleic	50.7
Malvalic	2.3
Sterculic	0.9
12,13-Epoxyoleic	1.6

Halphen colour reaction[83]

A solution of sulphur (1% in CS_2) was prepared for the Halphen test. Oil (1 ml) was taken in amyl alcohol (1 ml) and mixed with 1 ml of the above reagent. The reaction mixture was then heated on water bath for few minutes till CS_2 had boiled off. On keeping the test tube in an oil bath (110-115°C) for 1-2 hr, a red colour characteristic of cyclopropenoid fatty acid was developed.

HBr-titration[194] of the oil

The quantitation of cyclopropenoid and epoxy acids present in the oil was carried out by weighed amount of oil with 0.1N hydrogen bromide solution in glacial acetic acid using crystal

violet as indicator at two different temperatures to a bluish-green end point, that persists for 30 sec. At first the temperature was maintained at 3°C which gives the epoxy acid content. Then the temperature was raised to 55°C and the mixture was titrated again to a bluish-green end point to estimate the cycloprepenoid material present in the oil.

The percentage of the HBr-reactive acid (cycloprepenoid and epoxy) content was calculated by the following equation separately:

$$\% = \frac{29.45 \times N \times V}{\text{weight of the sample}}$$

where N = Normality; V = Volume of HBr consumed in titration.

Acetolysis of epoxide[110]

A 30 g portion of the oil was stirred overnight at room temperature with 120 ml of 10% sulphuric acid in 300 ml of glacial acetic acid. The mixture was diluted with distilled water and extracted with ethyl ether. The combined ether extracts were dried over anhydrous sodium sulphate and evaporated in a stream of nitrogen to yield a viscous oil.

Saponification of the recovered oil was effected by stirring with 0.8N alcoholic KOH overnight at room temperature. The unsaponifiable material was removed and the mixed fatty acids were recovered. Separation of these mixed fatty acids into oxygenated and non-oxygenated fractions was accomplished on layers

of silica gel 1.0 mm thick using petroleum ether-ethyl ether-acetic acid (90:20:1, v/v) as the developing solvent.

The same procedure of methanolysis was followed for *Vernonia anthelmintica* oil to obtain oxygenated fatty acid.

Preparation of $AgNO_3$-derivatives[115]

The non-oxygenated acids, separated from oxygenated acid by preparative TLC was esterified with ethereal diazomethane. A 200 mg portion of methyl esters was treated with 60 ml of absolute methanol saturated with silver nitrate. The reaction was allowed to proceed at room temperature with stirring for 24 hr. The normal methyl esters and the reaction products from cyclopropenes were recovered from the reaction mixture by adding 100 ml of distilled water and extracting with ethyl ether. The combined ether extracts were dried over anhydrous sodium sulphate and the solvent evaporated in the stream of nitrogen. Freshly prepared *S. foetida* esters was also treated with silver nitrate-methanol as above.

GLC analysis of $AgNO_3$-derivatives

The silver nitrate-methanol treated methyl esters of non-oxygenated fraction of *A. indicum* along with *S. foetida* ester derivatives were run into the GLC column under identical conditions to establish and quantitate the individual cyclopropenoid (sterculic and malvalic) acids present in *A. indicum* seed oil.

The column and conditions for GLC analysis were same as described in the section Analysis of Herbaceous Seed Oils. The GLC data are given in Table IV.

Purification of 12,13-dihydroxyoleic acid (II)

The procedure of acetolysis yielded 0.48 g of crude oxygenated fatty acid from 30 g of the oil. It was crystallised from acetone and petroleum ether-ethyl ether (3:1; v/v) to yield a crystalline product at low temperature melting at 53.5-54°C. An admixture with authentic threo-12,13-dihydroxyoleic acid (prepared from V. anthelmintica seed oil) had m.p. of 53.5-54°C. Co-chromatography on TLC plate also gave a single spot. The iodine value was 99.7 (lit.[126] 95.8), which was somewhat higher than that calculated for one double bond; there is evidently absorption of iodine due to reaction with the OH groups.

Analysis. Calc. for $C_{18}H_{34}O_4$: C, 68.78; H, 10.82.
Found: C, 68.67; H, 10.64%.
IR (CCl_4): 3480 (OH), 1720 cm^{-1} (COOH).

Hydrogenation of 12,13-dihydroxyoleic acid (II)

A 0.20 g portion of the dihydroxyoleic acid (II) on hydrogenation (Pt catalyst) in methanol consumed 0.97 mole equivalent of hydrogen and yielded 0.13 g of the product. On crystallisation from ethyl acetate it melted at 96-97°C. No depression of the m.p. was observed on admixture with an authentic

sample of 12,13-dihydroxystearic acid and it showed identical movement on direct TLC plate.

Analysis. Calc. for $C_{19}H_{36}O_4$: C, 68.35; H, 11.39.
Found: C, 69.21; H, 11.45%.

Permanganate-periodate oxidation[195] of unsaturated and saturated diols

A 0.10 g portion (each) and 0.127 g of potassium carbonate were dissolved in t-butyl alcohol (50 ml). To this mixture was added a solution of sodium metaperiodate (0.514 g) and potassium permanganate (1.0 ml of 0.057M) in 50 ml of water. The reaction mixture was stirred at ambient temperature for 24 hr, when reduced with sodium metabisulphite, acidified with HCl, and extracted with ethyl ether. Ethyl ether extracts after drying and evaporation yielded a semisolid mixture of products.

The products after methylation were analysed by GLC. The dihydroxyoleic acid (II) yielded hexanoic (IV) and azelaic (V) acids whereas the dihydroxystearic acid (III) gave hexanoic (IV) and dodecanedioic (VI) acids. The identity of the cleavage products was made by comparing their retention times with those of authentic samples obtained from V. anthelmintica oil.

A New Short-Chain Hydroxy Fatty Acid in *Anisochilus carnosus* (Labiatae) Seed Oil

Among the glyceride oils examined, one most unusual is from Labiatae (MINT FAMILY). Many secrete aromatic oils, and superficial oil glands are often visible on the leaves and flowers of Labiatae plants. These volatile aromatic essential oils account for a major share of the mint family's economic importance. Few Labiatae oils were reported to contain allenic fatty acids[72-77] in addition to normal fatty acids. The seed oils of the genus *Thymus*[73,146] and *Salvia nilotica*[147] of Labiatae were found to contain C_{17} and C_{18} hydroxy fatty acids.

No short-chain hydroxy fatty acid has been reported so far to occur in the seed oils. However, short-chain aliphatic hydroxy acids[196] containing one or more hydroxyl groups bound glycosidically to sugar moieties, have been reported in the seed oils of the plant family Convolvulaceae. Present work on *Anisochilus carnosus* (Hindi, Panjiri) shows it to contain a new short-chain monohydroxy unsaturated fatty acid (1.8%). The species *A. carnosus* unreported in the literature for its component fatty acids, is an aromatic herb representing the mint family and distributed in Western Himalayas (up to 9,000 ft) and Central and South India.

The hydroxy acid isolated from *A. carnosus* seed oil has been characterised as 9-hydroxy-*cis*-4-dodecenoic acid by IR, NMR, and mass studies of pure hydroxy methyl ester. The structure of

the hydroxy acid was further supported by its chemical transformations. Oil and seed characteristics were determined according to AOCS methods[1] and the results are summarised in Table V. The iodine value (I.V.) of the oil was found somewhat higher than the theoretical value. This observation is consistent with the literature[130] that hydroxy fatty acid-containing seed oils give higher I.V. apparently because of the involvement of the reagent with the hydroxy group.

Quantitation of component acids was made by GLC analysis of the methyl esters as their trimethylsilyl (TMS) derivative. The fatty acid composition given in Table VI shows the presence of hydroxy ester (1.8%) along with moderate proportions of normal fatty acids.

The concentrate of the hydroxy ester was obtained by silica gel column chromatography, using hexane-ethyl ether (90:10, v/v) as eluting solvent. The isolated ester showed single spot on analytical TLC plate. The UV spectrum of the ester showed no evidence for conjugation. Its IR spectrum (1% solution in CCl_4) showed no isolated trans unsaturation (965 cm^{-1}) but exhibited a broad band in the region 3500-3300 cm^{-1} indicative of hydroxyl group. The NMR spectrum (Figure 3) of the hydroxy ester has the following absorptions with proton integration, signal multiplicity, and probable assignments in parentheses: δ 0.89 (3H; t like; $\underline{CH}_3$-C-); 1.36 (9H; broad, s; -$\underline{CH}_2$-); 2.36 (6H; t; -$\underline{CH}_2$-COO and -$\underline{CH}_2$C-C=C-$\underline{CH}_2$-); 3.69 (3H; s; COO$\underline{CH}_3$); 5.35 (2H; m; -$\underline{CH}$=$\underline{CH}$-);

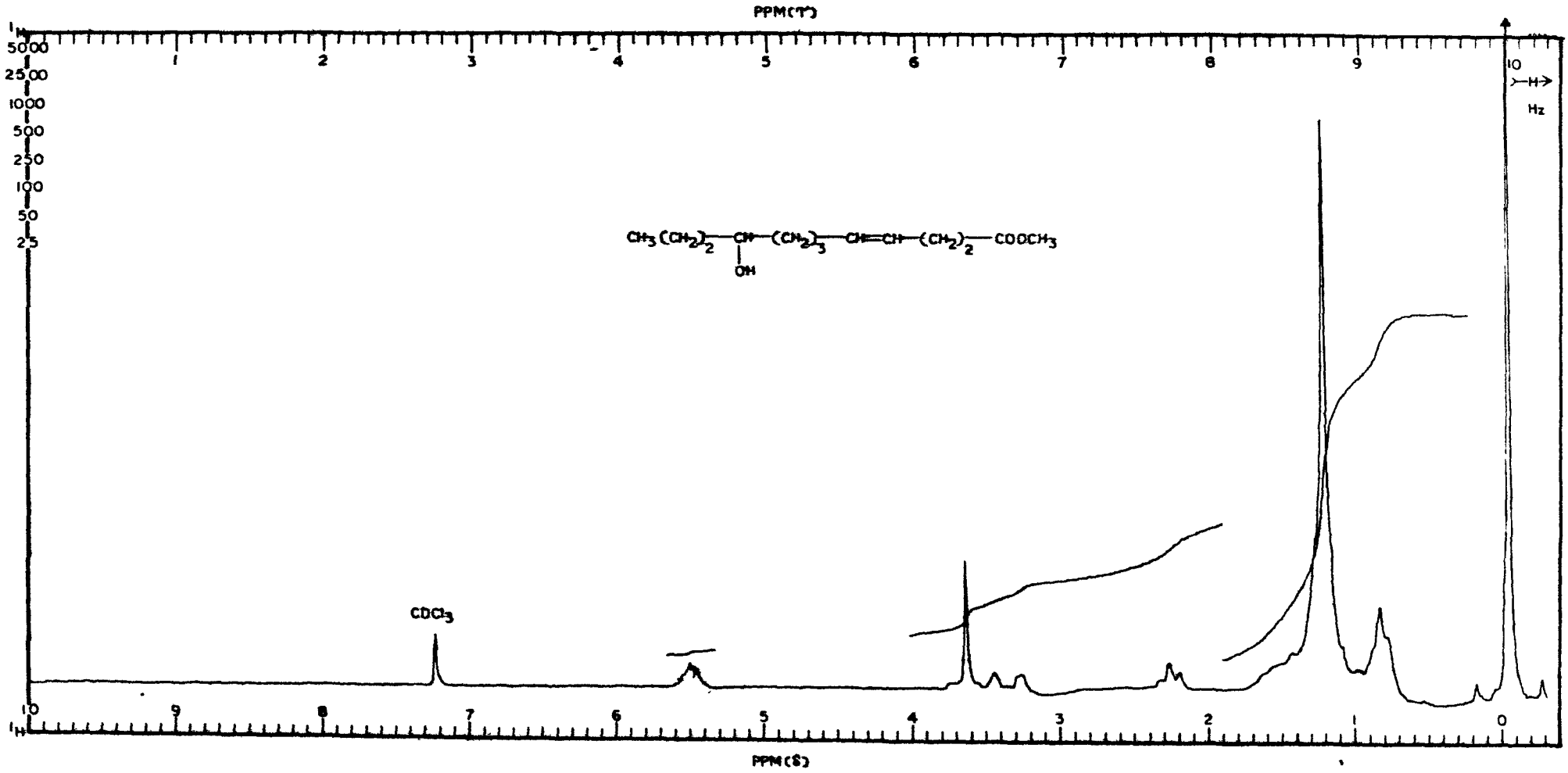


Fig 3. NMR SPECTRUM OF METHYL 9—HYDROXY—CIS—4—DODECENOATE (Ia)

3.31 (1H;broad,m;-CH-OH); and 3.47 (1H; broad, s; -CH-OH; disappeared on addition of D_2O). The spectrum had a strong peak at δ 2.35, characteristic of the protons C-2 and C-3 of a $\Delta 4$ unsaturated acid.[197] In conjunction with the multiplet for the double bonds at δ 5.35, this indicated the position of the double band at $\Delta 4$ in the fatty acid chain. From these data, the hydroxy ester was formulated as methyl 9-hydroxy-cis-4-dodecenoate. This formulation was further supported by mass spectrometry.

The gross structure of the hydroxy ester was determined by mass spectrometry (Figure 4). No molecular ion peak at m/e 238 ($C_{13}H_{24}O_3$) was observed. The highest mass peak was observed at m/e 227($M-H^+$). Other significant peaks at m/e 211, 196, 195, 169, 155, 141, 115, 113, 99, 97, 87, 85, 73, 71, 57, and 56 were considered to arise from the cleavages as indicated in the following Schemes. The mechanistic Schemes suggested are tentative.

m/e 211

The ion m/e 211 is derived from the molecular ion by the loss of mass unit 17, probably following the sequence shown in Scheme 3.

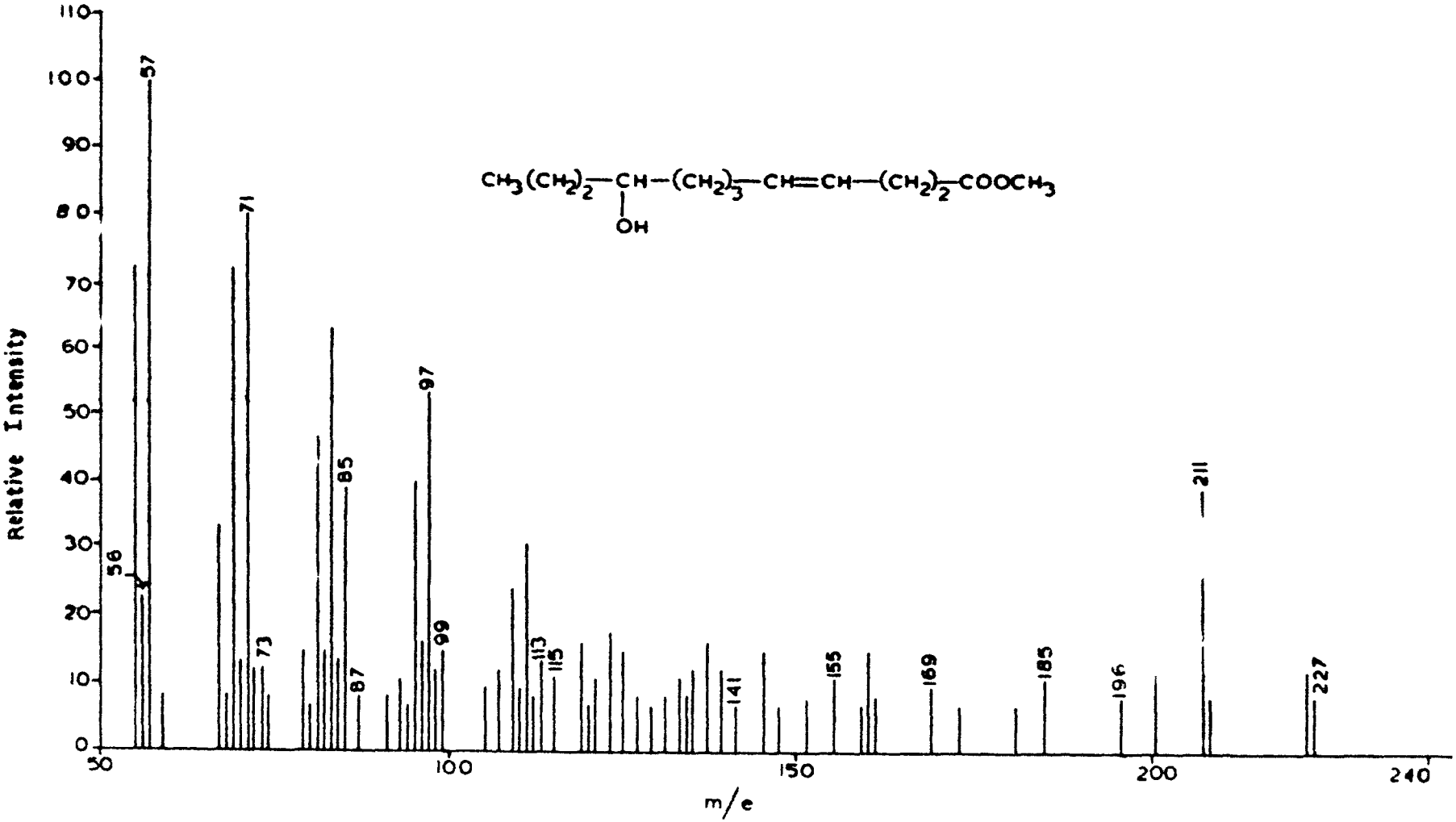
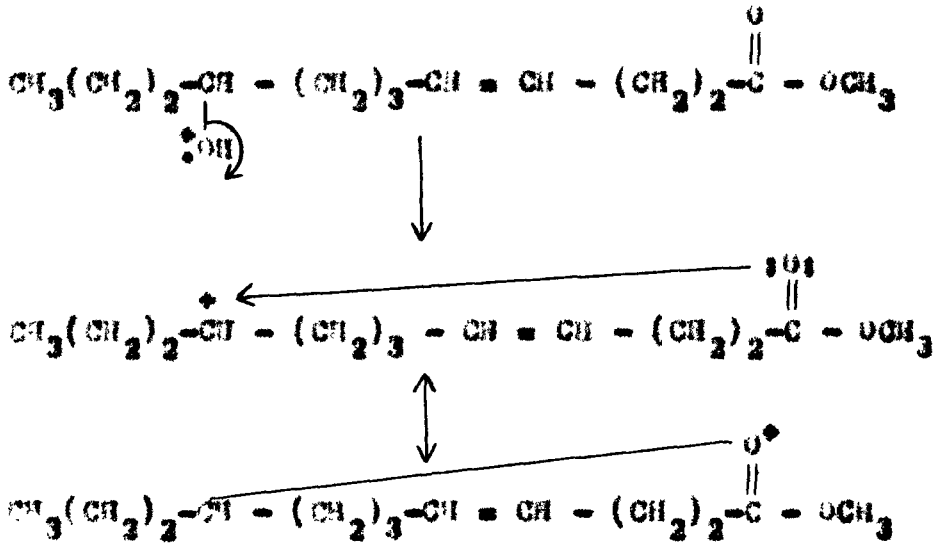


Fig. 4. Mass Spectrum of Methyl 9-hydroxy-cis-4-dodecenoate (Ia)

Scheme 3



m/e 211

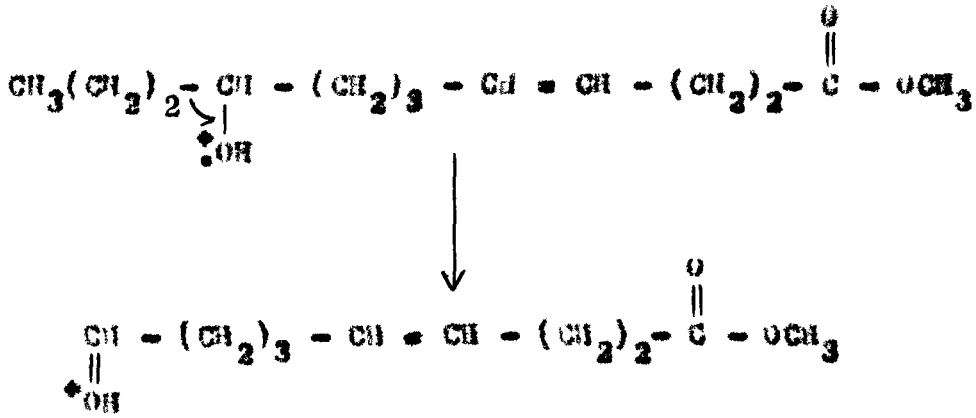
m/e 196 ($M-CH_3OH$)

The ion m/e 196 is originated by the loss of CH_3OH from the molecular ion m/e 228.

m/e 155

This fragment ion may arise from the molecular ion m/e 228 by cleavage between C-9 and C-10, probably following the mechanism shown in Scheme 4.

Scheme 4



m/e 185

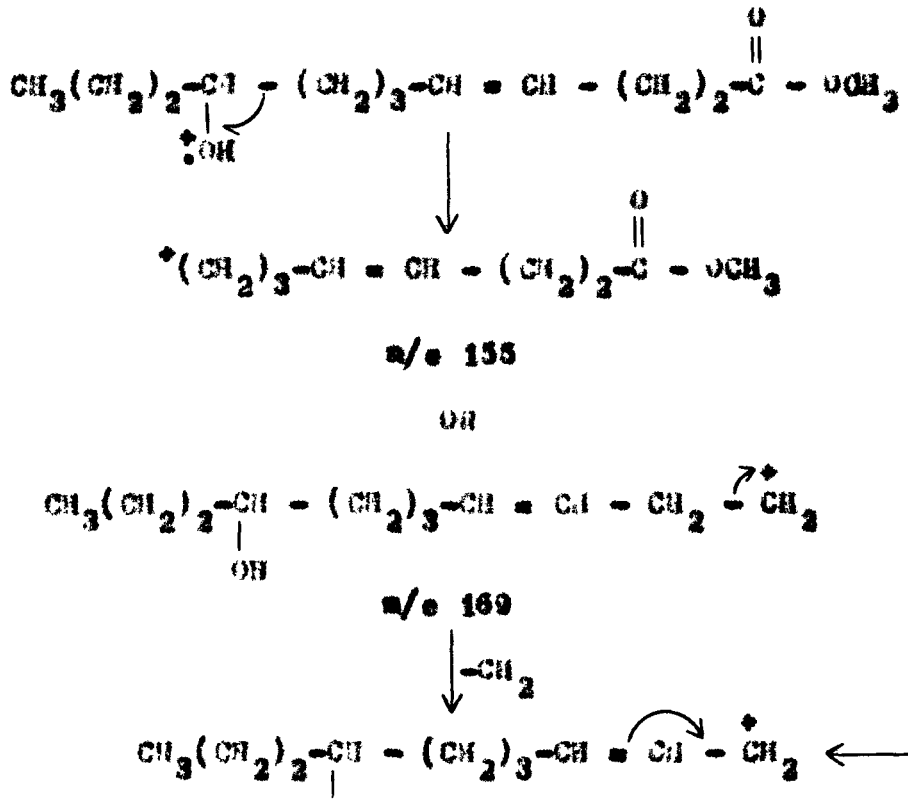
m/e 169 ($M-COOCH_3$)

The ion peak at m/e 169 may originate by the loss of $COOCH_3$ from the molecular ion m/e 228.

m/e 155

This fragment ion may arise from the molecular ion by cleavage between C-5 and C-6 and/or by the loss of CH_2 from the ion m/e 169 (Scheme 5).

Scheme 5



m/e 155

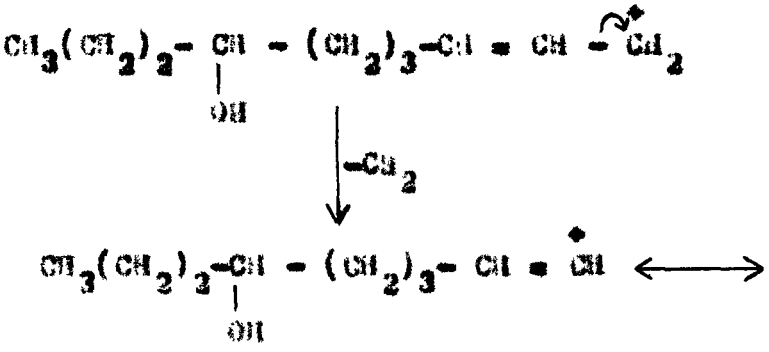
m/e 169

m/e 155

m/e 141

The fragment ion peak at m/e 141 results by the loss of CH_2 from the ion m/e 155 according to Scheme 6.

Scheme 6

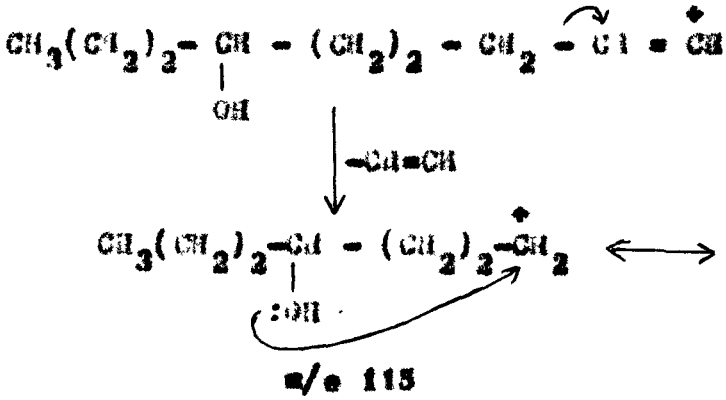


m/e 141

m/e 115

This ion peak can be shown to arise by the loss of CH=CH from the ion m/e 141 (Scheme 7).

Scheme 7

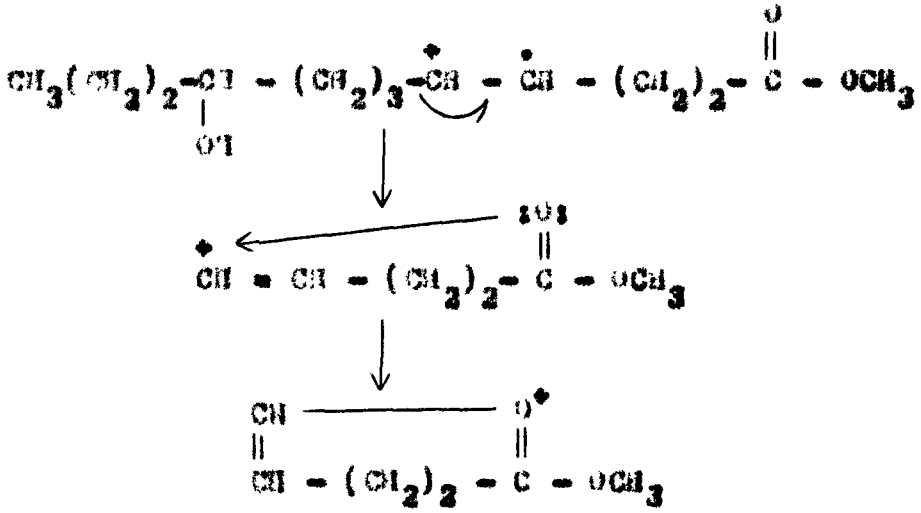


m/e 115

m/e 113

This fragment ion could possibly be obtained from the molecular ion according to Scheme 8.

Scheme 8

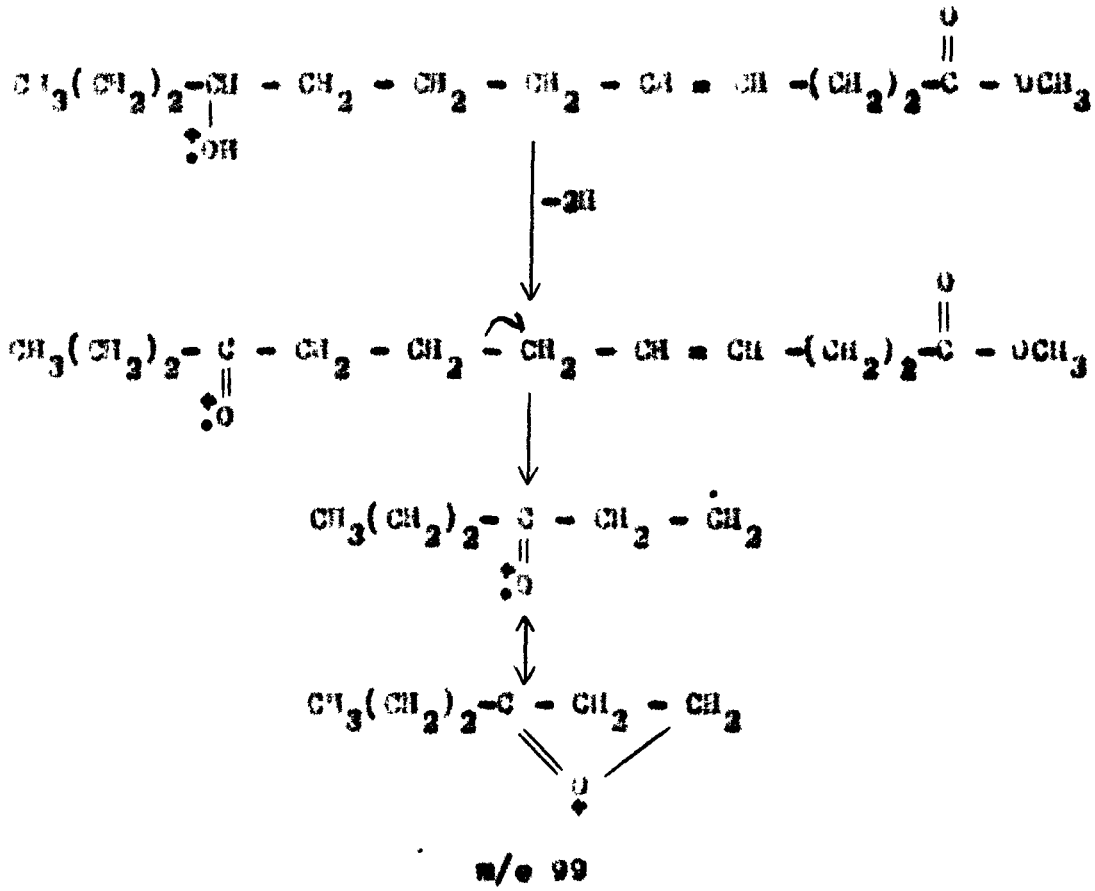


m/e 113

m/e 99

This ion can be shown to arise from the molecular ion, probably following the way depicted in Scheme 9.

Scheme 9



m/e 99

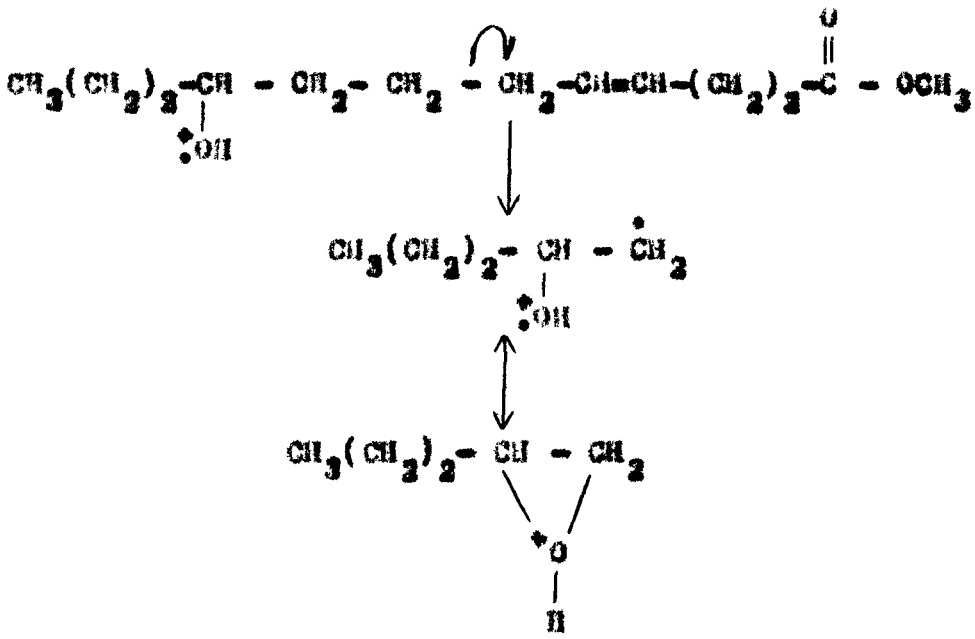
m/e 97

The ion peak m/e 97 may originate by the loss of a water molecule from the ion m/e 115.

m/e 97

This ion peak can be shown to arise from the molecular ion according to Scheme 10.

Scheme 10



m/e 97

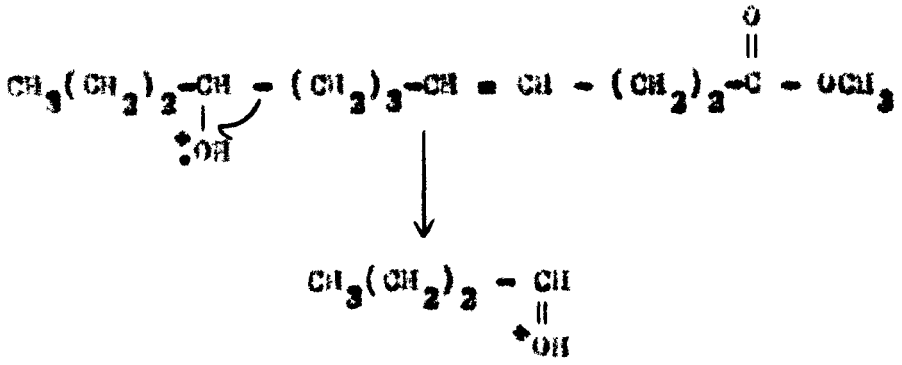
m/e 95

The fragment ion peak at m/e 95 results by the loss of CH_2 from the ion m/e 99.

m/e 73

This fragment ion arises from the molecular ion by cleavage between C-3 and C-9 according to Scheme 11.

Scheme 11

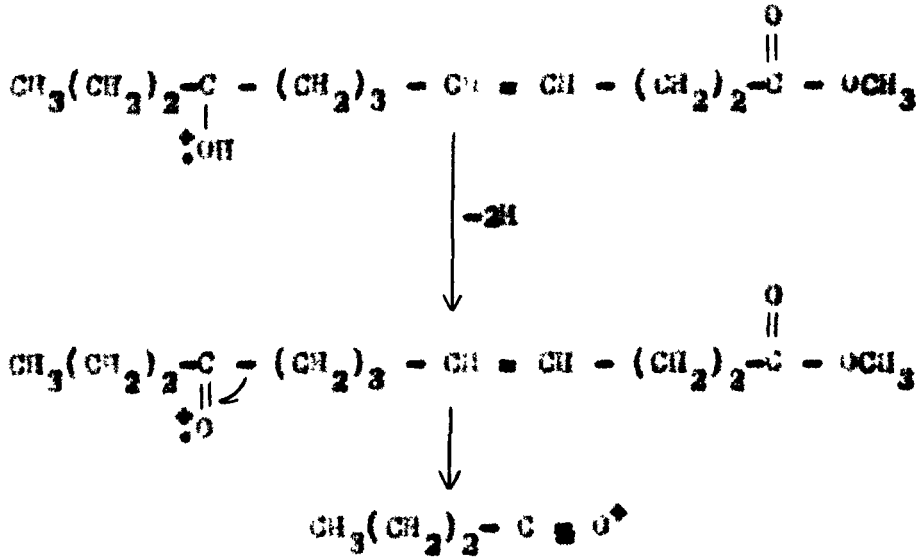


m/e 73

m/e 71

This ion can be shown to arise from the molecular ion, probably following the way depicted in Scheme 12.

Scheme 12

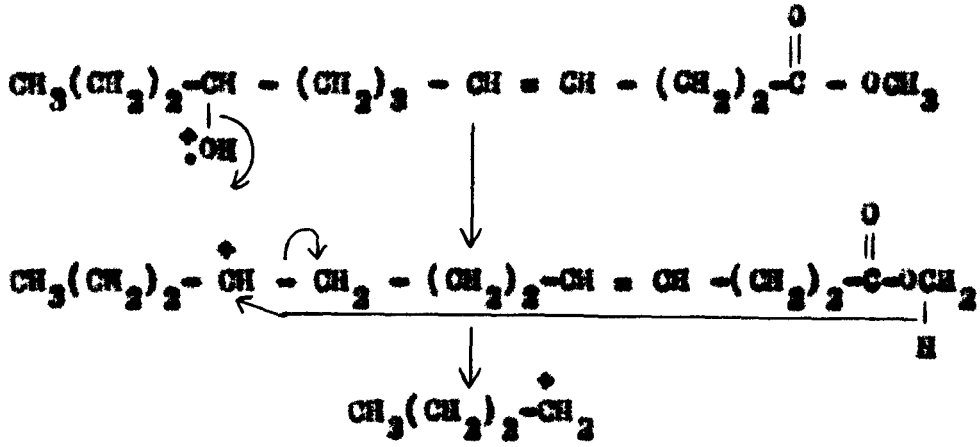


m/e 71

m/e 57

The fragment ion peak m/e 57 constitutes the base peak of the spectrum. This ion may arise from the molecular ion, probably following the sequence shown in Scheme 13.

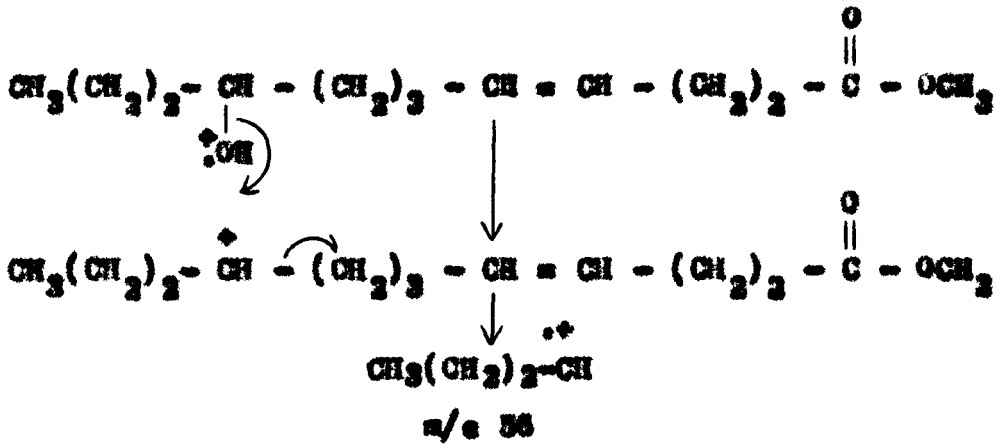
Scheme 13



m/e 56

This ion can be shown to arise from the molecular ion according to Scheme 14.

Scheme 14

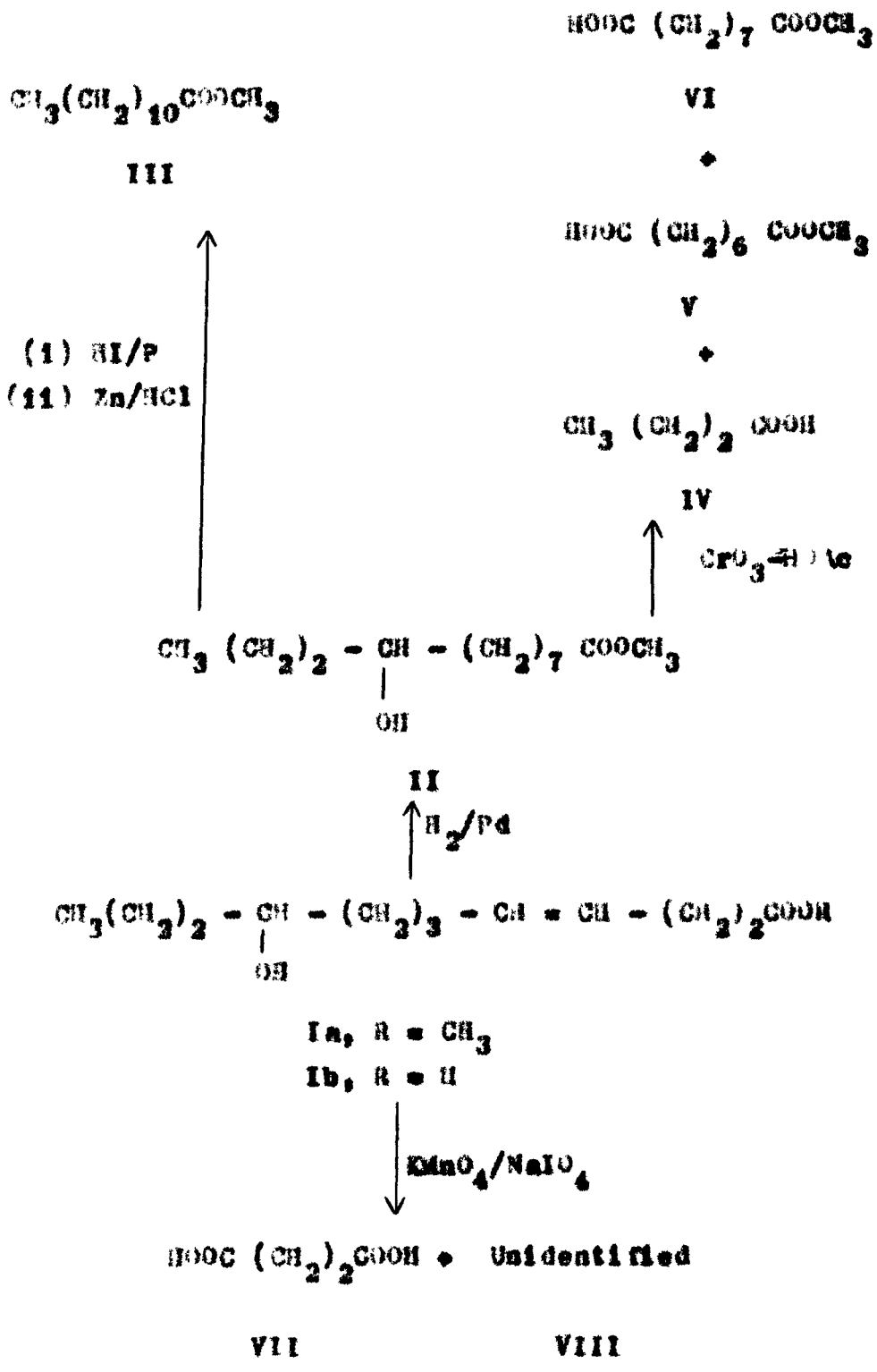


The diagnostic ions discussed above placed the hydroxyl group at C-9 and the double bond in between C-4 and C-5 of 12 carbon chain.

The identity of the structure, methyl 9-hydroxy-cis-4-dodecenoate (Ia) is also based upon its chemical transformations (Scheme 15). The hydrogenation of Ia furnished saturated hydroxy ester (II) which was analysed for $C_{13}H_{26}O_3$ and had an IR absorption at 3450 cm^{-1} (OH); IR showed no trans absorption. Reductive removal of hydroxyl group in II by hydrogen iodide and phosphorus followed by zinc and hydrochloric acid[193] furnished III which was identified as methyl laurate by GLC and elemental analysis. This established a normal C_{12} skeleton for I. The structure of saturated hydroxy ester (II) was established by oxidative cleavage with chromium trioxide in acetic acid.[163] The cleavage products obtained, half esters of octanedioic (V) and nonanedioic (VI) acids in nearly equal amounts along with butyric acid (IV), placed the hydroxyl at 9 position of a normal C_{12} skeleton. Hydroxy acid (Ib) on permanganate-periodate cleavage[193] yielded two fragments. One of these was identified as succinic acid (VII) by GLC analysis. This identification showed that the double bond was in the $\Delta 4$ position.

The above acid having double bond in the 4-position isolated from A. carnosus seed oil may be of special significance in the study of biosynthesis of unsaturated acids.

Scheme 15


HOOC (CH2)7 COOCH3
VI
HOOC (CH2)6 COOCH3
V
CH3 (CH2)2 COOH
IV
CrO3-H2SO4
(I) HI/P
(II) Zn/HCl
CH3 (CH2)2 - CH - (CH2)7 COOCH3
OH
II
H2/Pd
CH3(CH2)2 - CH - (CH2)3 - CH = CH - (CH2)2COOH
OH
Ia, R = CH3
Ib, R = H
KMnO4/NaIO4
HOOC (CH2)2COOH + Unidentified
VII
VIII

EXPERIMENTAL PROCEDURES

Preliminary analysis of oil and methyl esters

Coarsely grounded seeds of *A. indicum* were extracted in Soxhlet apparatus with petroleum ether (40-60°C). The bulk of the solvent was evaporated on steam bath and the remainder was removed in vacuo with a rotating evaporator. The analytical values of the oil and seeds were determined according to the procedures recommended by the AOCS[1] and data are summarised in Table V.

The oil was saponified by refluxing for 1 hr with 1N KOH in ethanol. The solution was then cooled, diluted with water, and the unsaponifiables were extracted with ethyl ether. The alkaline liquor was acidified with dil.HCl and extracted with ethyl ether. The extracts was demineralised by washing with water, and the mixed fatty acids were obtained by evaporating the solvent at elevated temperature. The mixed fatty acids on treatment with ethereal diazomethane[199] furnished total methyl esters. Direct TLC of the esters showed a spot (R_f 0.24) for hydroxy component, the other components moving with the solvent front. Argentation TLC revealed the presence of saturates, monoene, diene, triene, and a small low moving spot for hydroxy component.

Silylation of methyl esters[195]

To 0.01 g portion of the total methyl esters was added pyridine (1 ml), hexamethyldisilazane (0.3 ml) and trimethylchlorosilane (0.1 ml). The mixture was shaken for 30 sec and allowed to stand for 5 min. Then hexane (5 ml) and water (5 ml) were added and the whole shaken. The hexane layer was removed and the aqueous layer washed twice more with 5 ml portions of hexane. The combined hexane layers were dried over anhydrous sodium sulphate, then the solvent was removed under vacuo until the odour of pyridine has gone. The derivative needed no further purification and was subjected as such into the GLC column. The result has been summarised in Table VI.

Enrichment of hydroxy ester (Ia)

The mixed methyl esters were fractionated by silica gel column chromatography. Elution with hexane—ethyl ether (98:2, v/v)

TABLE V

Aniseohilus carnosus Seeds and Oil

Property	Value
Composition of seeds (%)	
Oil	15.6
Protein	34.7
Moisture	3.6
Oil characteristics	
Refractive index n_D^{40}	1.4652
Iodine value (Wijs)	70.4
Saponification value	177.2
Unsaponifiable matter (%)	3.2

TABLE VI

Fatty Acid Profile of *Aniseohilus carnosus* Seed Oil

Fatty Acid	Area % by GLC
14:0	14.0
16:0	37.7
18:0	2.3
18:1	23.2
18:2	12.6
18:3	8.4
9-Hydroxy-*cis*-4-dodecenoic	1.8

(15 ml fractions collected) gave the normal ester and subsequent elution with hexane-ethyl ether (90:10, v/v) gave the hydroxy ester. The isolated hydroxy ester showed single spot on TLC plate. The iodine value indicated the presence of one double bond. Analysis. Calc. for $C_{13}H_{24}O_3$: C, 68.42; H, 10.53. Found: C, 68.36; H, 10.49%. IR (CCl_4): 3500-3300 (OH), 1740 (COOMe), and 1090 cm^{-1} (C-O). NMR: δ 0.89 (3H; t like; CH_3-C-); 1.36 (8H; broad s; -CH_2-); 2.36 (6H; t; -CH_2-COO and -H_2C-C≡C-CH_2-); 3.68 (3H; s; $COOCH_3$); 5.35 (2H; m; -CH=CH-); 3.31 (1H; broad, m; CH-OH); and 3.47 (1H; broad s; CH-OH; disappeared on addition of D_2O). Mass: m/e 227 (7.5), 211 (30.9), 196 (3.6), 185 (11), 169 (9), 155 (10.2), 141 (6.9), 115 (10.4), 113 (13), 99 (14.7), 97 (53.1), 87 (8.6), 85 (39.1), 73 (12.4), 71 (79.3), 57 (100), and 56 (22.6).

Hydrogenation of hydroxy ester (Ia)

Hydroxy ester (Ia, 0.24g) dissolved in ethyl acetate (2 ml) was hydrogenated at 45 Psi for 12 hr with palladium carbon catalyst. After filtration and evaporation, hydrogenated ester (II) was obtained. Analysis. Calc. for $C_{13}H_{26}O_3$: C, 67.83; H, 11.30. Found: C, 67.78; H, 11.23%. IR (CCl_4): 3448 (OH), 1740 cm^{-1} ($COOCH_3$).

Reductive removal of hydroxyl in saturated hydroxy ester (II)[193]

A 0.1 g portion of II was refluxed for 17 hr with red phosphorus (0.05 g) and hydriodic acid (3 ml; sp.gr. 1.7). The mixture was diluted with water and extracted repeatedly with ethyl

ether. Combined ethyl ether extracts were washed with 5% sodium metabisulphite, then dried over anhydrous sodium sulphate. A colourless oil (0.08 g), thus obtained was reduced by heating at reflux 4 hr with granular zinc (0.20 g), methanol (5 ml) and conc. HCl (1 ml). The mixture was then diluted with water and extracted with ethyl ether. Drying and evaporation of ethyl ether yielded semi solid ester (III) (0.068 g). GLC analysis indicated the compound to be methyl laurate.

Chromium trioxide oxidation of saturated hydroxy ester (II)

A 0.035 g portion of II was dissolved in 3 ml of glacial acetic acid. To this mixture was added, dropwise and with continuous stirring, a solution of 0.35 g of chromium trioxide, 2.5 ml of acetic acid, and 0.3 ml of water. The mixture was stirred at room temperature for 2.5 hr and diluted with 5 ml of water; the products were recovered by extraction with ethyl ether. The recovered acids were esterified with diazomethane[199] and GLC analysis gave the following results: butyric (IV)(16.6%), octanedioic (V)(23.8%), nonanedioic (VI)(22.4%) acids, and an unknown acid. The identity of the cleavage products was made by comparing their retention times with those of authentic samples.

Permanganate-periodate cleavage[195] of hydroxy acid (Ib)

A 0.093 g portion of Ib was subjected to oxidative cleavage following the procedure mentioned previously in the section

Characterisation of $KMnO_4$-reactive Acids in *A. indicum* Seed Oil. The concentrate of the cleavage products after methylation with diazomethane was analysed by GLC. One of the products was identified as succinic acid (VII) by comparing its retention time with that of authentic sample on the gas chromatogram. The other component (VIII) could not be identified.

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P A R T - II

REACTIONS OF ACETYLENIC FATTY ACIDS

THEORETICAL

During the past 20 years, there has been a remarkable resurgence in the interest and research effort devoted to a study of the chemistry of fatty acids and their derivatives for use as raw-materials in oil-based industries. In general, the reactions of fatty acids can be divided into two broad groups: (a) those involving the hydrocarbon chain, and (b) the reaction of the terminal carboxyl group. Fatty acids generally undergo all classical reactions of organic chemistry. The modern researches are aimed at the studies on non-classical reactions of fatty acids to synthesise new fatty acid derivatives.

Acetylenic Fatty Acids

It was believed at one time that the acetylenic bond did not occur in natural products. This belief has been dispelled by the discovery of several hundred acetylenic compounds in seed oils, fungi, and the essential oils of higher plants. The acetylenic fatty acids mostly occur in seed oils of Santalaceae and Olacaceae families. The mechanism by which the triple bond originates in nature has been studied by Bu'Lock.[1,2]

The known naturally occurring acetylenic fatty acids comprise a group of acids having hydrocarbon chains containing one or more triple bonds, double and triple bonds, double and triple bonds and a hydroxyl group. In addition to several

natural acids, an extensive number of related acids have been prepared synthetically. More extensive reviews of the natural and synthetic acids were published by Meade,[3] Smith,[4] Hopkins,[5] and Bohlmann,[6] for the former; and Raphael,[7] Meade,[3] and Markley[8] for the latter. The most recent review on synthesis of unsaturated fatty acids written by DeJarlais[9a] deals with various synthetic methods used for acetylenic acids.

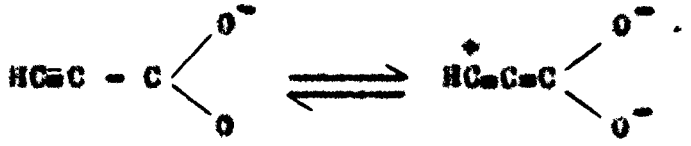
Acetylenic acids show the chemical and physical properties of fatty acids, plus those of acetylenic compounds. The octadecynoic acids are the best known acetylenic acids and constitute the most complete series of positional isomers of this class of compounds. Of the sixteen theoretically possible positional isomers, six have been synthesized[8] and a seventh isomer (tariric) has been found as a constituent of natural fats.[10] 9-Octadecynoic (stearolic) acid has never been found as a constituent of natural fats but is of more than ordinary interest because of its structural similarity to oleic acid. Nine positional isomers of undecynoic acid are theoretically possible. None has been observed as a constituent of natural fats but several of them have been synthesized.[8]

Bohlmann *et al.*[6] remarked that crepenynic acid is "perhaps the most important acetylenic fatty acid". Widespread interest in the biochemistry of this acetylenic analogue of linoleic acid has been evidenced in several ways. Its role in

the biosynthesis of more highly unsaturated acetylenes has been reported.[11] The unique structure and chemistry of crepenynic acid makes it a potential source of cyclic acids, either saturated or aromatic, for coatings, resins, and low-temperature lubricants.[12,13]

Reactions

The acetylenic group greatly increases the strength of a neighbouring carboxyl group, propiolic acid (HC≡C-COOH) having a dissociation constant 10^3 greater than propionic acid. This effect is limited to acid groups conjugated to acetylene, and has been ascribed to a resonance with an allenic structure.



The properties of the carboxyl group are otherwise normal.

The reactivity of α-hydroxyacetylenic [-C≡C-CH(OH)-] acids is influenced by the strong electron-withdrawing character of the triple bond. This electron-withdrawing property deters carbonium ion formation on the α-carbon thus inhibiting acid catalysed dehydration via an Si mechanism. The chemistry of the enynol [-C≡C-CH=CH-CH(OH)-] grouping[14] evidently differs from that of dienol [-C=C-CH=CH-CH(OH)-] grouping in certain respects. The enynol system evidently behaves chemically as an vinylogue of an α-hydroxyacetylene rather than as an ordinary

allylic alcohol or as a conjugated dienol. A carbonium ion derived from a conjugated enynol system (I) appears to be less readily formed than one from a corresponding dienol (II). The

I II

difference in the reactivity of the two systems can be rationalised by assuming that the energy barrier for reactions proceeding through I is appreciably greater than the corresponding energy barrier for reactions proceeding through II. Thus reactions (etherification, rearrangement, or dehydration) requiring I as an intermediate will be much slower than the corresponding reactions requiring II. With each system, the etherification would be the fastest of the three competing reactions and the dehydration the slowest.

Partial catalytic and chemical reduction of acetylenic fatty acids provides access to cis- and trans-ethylenically unsaturated acids. The semi-hydrogenation of acetylenic acids by heterogenous catalysts is usually carried out with the goal of synthesising the corresponding unsaturated fatty acids. The mechanisms and applications of this reaction have been discussed in comprehensive reviews published recently.[9b,15,16] Tritium labeled polyunsaturates are usually prepared by selective reduction of the appropriate polyacetylenic fatty acid.[17-19]

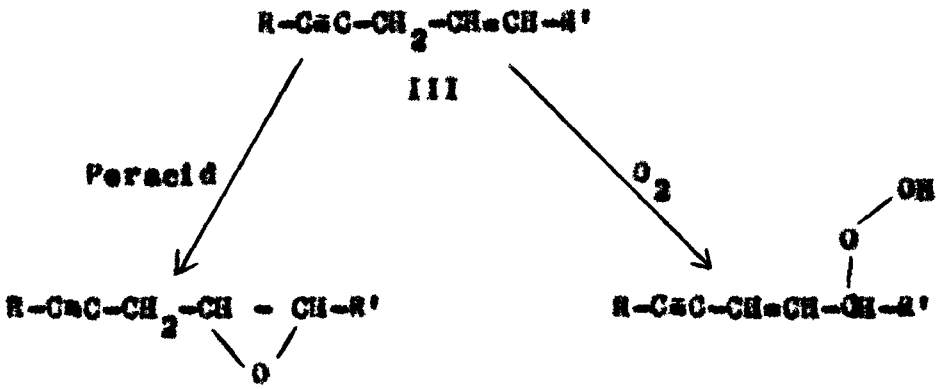
Mid-chain acetylenic acids are stable to lithium aluminium hydride, methyl stearolate giving stearolyl alcohol. Undecyl alcohol was obtained by the reduction of methyl undecynoate with sodium (yield 86%) or lithium aluminium hydride (yield 95%).[20]

Halogen reacts somewhat slowly: bromine adds 50,000 times faster to oleic than to stearolic acid, and further addition is slower still, requiring activation. Addition of iodine, which is very incomplete to olefinic acids, is complete with acetylenic acids, proceeding readily in warm acetic acid solution in presence of ferrous iron to give stable crystalline diiodides which are useful for identification, and have been proposed as X-ray contrast media. Addition of halogen to undecynoic, tariric, stearolic, behenolic, and similar acids to yield corresponding dihaloethylenic acids has been reported.[21-23] Hydrogen halides add doubly to acetylenic acids giving the haloolefinic and then presumably the gem-dihalo acids, vicinal diiodides being very unstable. Addition of hydrogen bromide to 10-undecynoic acid and hydrogen iodide to stearolic acid[21-23] have been published.

Peracetic and performic acids act very slowly on simple acids, giving chain cleavage and a little hydration to the ketone: this unreactivity is very marked with ynoic acids, where addition to the double bond occurs normally, with very little attack on the acetylenic linkages. This is a valuable weapon in constitutional studies. Consequently, when methyl crepenynate

(III) is reacted with m-chloroperbenzoic acid (Scheme 1), it is readily converted into a monoepoxide (IV) in which the acetylenic linkage is preserved.[24,25] Structure III yields an autoxidation product (V) analogous to the familiar linoleate hydroperoxide,[26] but with a conjugated enyne structure.

Scheme 1



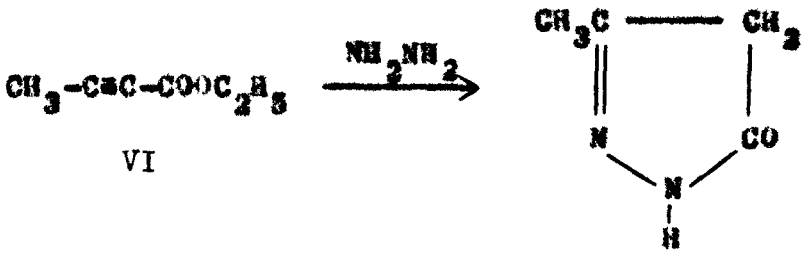
Hydration of acetylenic acids is accomplished by means of conc. sulphuric acid. The reaction may be represented as

$$-C\equiv C- + H_2O \longrightarrow -CH=C(OH)- \longrightarrow -CH_2-CO-$$

Hydration[27] of 9-stearolic and 13-behenolic acids with conc. H_2SO_4 produces almost equal amounts of the corresponding isomeric oxo acids. On the other hand 6-stearolic acid yields a mixture of 74.5% of 7-oxo- and 25.5% of 8-oxostearic acids, perhaps because of the proximity of the acetylenic bond to the carboxyl group.

10-Undecynoic acid yields 10-oxoundecanoic acid as the sole product thus exhibiting Markownikoff's addition during hydration of a terminal triple bond.[27]

The ester of acetylenic acids, with the exception of α,β-unsaturated acids, can be converted to hydrazides.[28] Ethyl stearolate and ethyl undecynoate readily give hydrazides. α,β-Acetylenic esters react with hydrazine to form pyranolones; for example, ethyl tetrolate (VI) gives 3-methyl-5-pyranolone (VII).

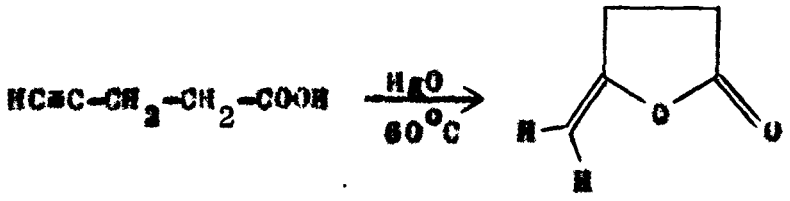


VI

VII

α-Acetylenic acids which have a γ-hydrogen atom are isomerised by sodamide in ammonia to the corresponding allenic acid in quantitative yield.[29]

Various γ-methylenebutyrolactones have been synthesised in good yield by the cyclisation of acetylenic acids in the presence of a catalytic amount of mercury (II) oxide.[30]



Oxymercuration reaction of acetylenic esters gives oxo and hydroxy esters when demercurated with hydrochloric acid and sodium borohydride, respectively. Lam and Jie[31,32] have reported solvomercuration-demercuration of acetylenic esters. Recently Ahmad[33a] from author's laboratory obtained 9- and 10-oxoundecanoic acids, instead hydroxy acids, by the reduction of the acetoxymercuri intermediate of 9-undecynoic acid with sodium borohydride.

Currently the lipid chemists have been engaged in studies designed to learn more about the new and interesting reactions of acetylenic fatty acids, which provide new route to the synthesis of a variety of fatty compounds. The recent advances in chromatographic methods of separation and spectroscopic methods of structure determination have led to the work on the kinetics of reactions and the use of physico-chemical data in solving the mechanistic problems of fatty acids. Thus, the conventional elemental data, melting point determination, and derivatisation of reaction products are no longer the sole criteria for structure determination. The spectral methods — particularly NMR and mass spectroscopies — which have most changed the behaviour pattern of lipid chemists and biochemists in recent years, are now briefly discussed.

Nuclear Magnetic Resonance (NMR)

A number of reviews[34-37] on the NMR spectra of fatty acids have appeared in the literature. The general pattern of the signals appeared for the protons in long-chain esters is as follows. The ω-methyl signal usually appears as a distorted triplet at δ 0.88-0.89 except when disturbed by an appropriately placed functional group. The chain methylene protons give a broad band at δ 1.33. The C-2 protons (α to ester carbonyl) give a very distinctive triplet at δ 2.31 which is shifted

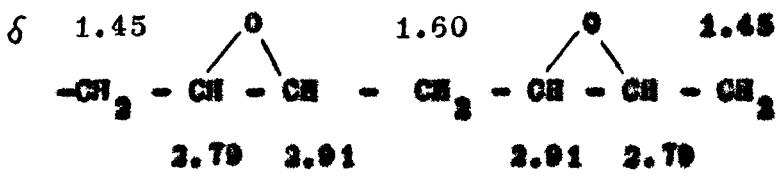
downfield differently due to different functional groups present in the chain. A sharp singlet at δ 3.60-3.70 appears for the protons of ester methyl.

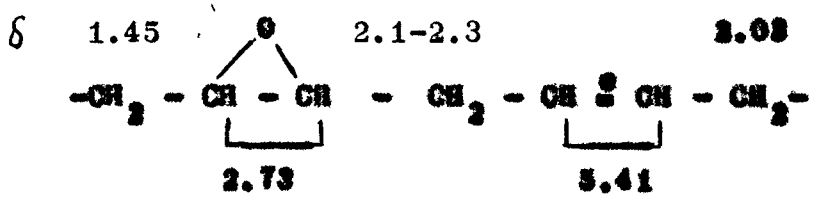
Olefinic protons normally give signals at δ 5.30 (*cis*) and δ 5.32 (*trans*).[38] The 'J' value for *trans* olefinic protons are regularly higher than those corresponding to *cis* protons. Fatty acids with *trans* α,β -unsaturation have coupling constant at ~ 16 Hz. Methyl *trans*-2-hexadecenoate has separate signals for the two olefinic protons. The spectrum gives doublet of doublet centred at δ 6.9 (J = 15 and 5 Hz) ascribable to a proton β to ester carbonyl and a doublet at δ 6.0 (J = 15 Hz) ascribable to a proton α to ester carbonyl. The NMR can also be used to quantitate the *cis*-*trans* ratio by derivatisation. Schaumburg[39] has given a detailed account of the NMR spectra of the ureourated adduct of unsaturated fatty acids. The signal resulting from the allylic protons is appeared at δ 1.98-2.14, *cis* and δ 1.93-2.00 *trans*. This signal is frequently complicated[40] if functional groups are attached to the chain. In such cases the two sets of allylic protons have different chemical shifts and one pair is sometimes sufficiently deshielded to produce a signal which overlaps with that resulting from the C-2 protons. The situation may be further complicated by non-equivalence of the allylic protons which produce the overlapping signal.

NMR spectra show the presence of conjugated bonds by a widely split signal in the region of δ 6.0, although there can be some interference from α,β-unsaturation, a terminal double bond, or aromatic rings, all of which give signal at about the same region. Methylene protons (propargylic) adjacent to one triple bond give a signal at δ 2.06-2.14. The four propargylic protons adjacent to a conjugated diyne unit appear a little downfield at δ 2.22. Methylene protons sandwiched between two triple bonds ($-C\equiv C-CH_2-C\equiv C-$) appear at δ 2.97-3.00 for the diynes. The group $-C\equiv C-CH_2-CH_2-C\equiv C-$ which occurs in the diynoic acids gives a distinctive signal at δ 2.24-2.35 arising from the four internal propargylic protons which are under the combined influence (α and β). Gunstone *et al.*[41,42] studied the spectra of a number of octadecadiynoic acids and of the derived *cis*, *cis*- and *trans*, *trans*-octadecadienoates using a 100 MHz instrument. From the chemical shifts of propargylic and allylic protons, these workers concluded that spectral differences between these compounds result from the relative position and strength of various deshielding influences. Deshielding is largely confined to protons on the adjacent carbon atoms (α); the effect on methylene groups further removed (β or γ) is very small. The effectiveness of these deshielding centres is given by the sequence,[42] $-COOH > -COOMe > -C\equiv C-C\equiv C- > -CH\overset{2}{=}CH-CH\overset{2}{=}CH- > -C\equiv C- > -CH\overset{1}{=}CH-CH\overset{1}{=}CH- > -CH\overset{2}{=}CH- > -CH\overset{1}{=}CH-$. From

careful study of the 220 MHz spectra of a large number of appropriate compounds Frost et al.[38,43] concluded that deshielding effects of functional groups along an alkyl chain may affect hydrogen atoms attached to carbon atoms up to five or six carbon centres from the functional group.

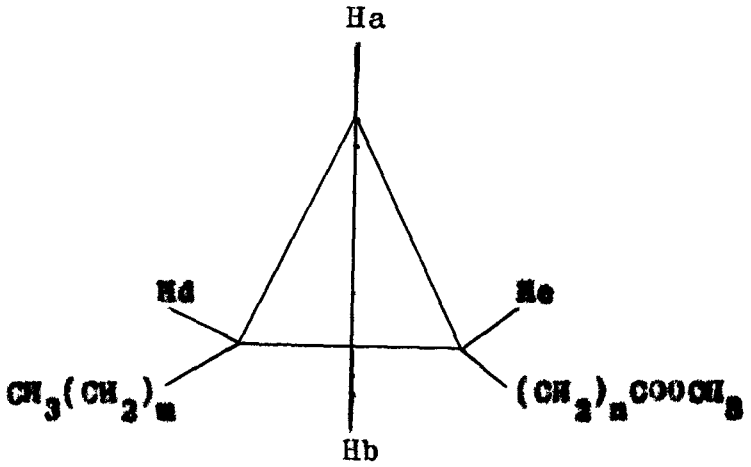
In the case of oxygenated acids, hydroxy acids give rise to two separate signals along with other usual signals in NMR which represent the -O<u>H</u> proton and -C<u>H</u>(OH)- proton. The signal of the proton of the hydroxyl group is indistinct which varies due to hydrogen bonding effects and can be eliminated by a drop of D_2O. The signal due to methine proton at δ 3.60 is quite characteristic. When the hydroxy esters are acetylated, the acetoxy protons give rise to sharp signal at δ 2.10. Keto groups influence the α-methylene protons which produce a signal similar to that for protons adjacent to a ester carbonyl group. Unperturbed epoxidic protons give ^1H NMR signals at δ 2.70 (J = 3.5-5.0 Hz) and δ 2.45 (J = 0.5-2.5 Hz) for <u>cis</u> and <u>trans</u> isomers, respectively. The epoxide system also exerts a deshielding effect on CH_3 and CH_2 protons along an alkyl chain.[40] Typical values for a diepoxide (methyl <u>cis</u>-9,10; <u>cis</u>-12,13-diepoxystearate) and for an unsaturated epoxide (methyl <u>cis</u>-12,13-epoxyoleate) are:





Similarly two signals at δ 2.65 and 2.52 for trans, trans diepoxides and four signals at δ 2.91, 2.78, 2.86 and 2.55 for cis, trans diepoxides are appeared.[40]

The olefinic protons in a cyclopentene ring give signal at δ 5.7 in NMR. Pawlowski et al.[44] have described the use of NMR spectroscopy as a rapid, simple, and quantitative method of analysis for the cyclopropenoid function in lipids. The NMR spectrum of cis-1,2-dialkyl cyclopropenoid ester is characterised by a multiplet at δ -0.3 (1 proton) assigned to the proton Hb cis to the two alkyl substituents and a broad band at δ 0.6



(3 protons) arising from the Ha, Hc, and Hd protons. The corresponding trans cyclopropane isomer has four protons band centered at δ 0.2 with fine structure between δ 0.6 and -0.1 in its spectrum. The bands at δ 0.6 and -0.3 occur in the spectra of the cis-5,6- to 14,15-methyleneoctadecanoates though the spectra of the remaining isomers have distinguishing features.[45]

In recent years few NMR techniques have been developed which have found application in the structure elucidation of compounds of complex structure. Among these are: (a) application of decoupling (double resonance or double irradiation) technique to simplify complex signals and to identify related protons, (b) the use of chemical shift reagents (CSR), (c) ^{13}C NMR, (d) chemically induced dynamic polarisation (CIDNP)[46] technique, and (e) ^{13}C Fourier transform NMR (FT NMR).[47]

Application of ^{13}C NMR studies[48-53] of saturated, olefinic, and acetylenic esters have been reported recently. Rakoff et al.[54] have reported ^{13}C NMR spectra of different mono- and dihydroxy saturated and unsaturated fatty acid methyl esters. ^{13}C FT NMR is a simple, fast, and non-destructive method which has been successfully used in the direct characterisation of fatty acids and sugars in seeds.[47]

Mass Spectrometry (MS)

Mass spectrometry is one of the most widely used spectroscopic techniques now available for the structural determination of an ever increasing variety of natural products. Within these areas, fatty acid esters occupied a unique position, in that they represent one of the earliest and most comprehensively studied classes of natural products to be investigated. The early and systematic work of Ryhage and Stenhagen[58] in Sweden has provided an extensive description of the mass spectra of fatty acids and their esters.

The use of mass spectrometry for determining the structure of fatty acids — now increasingly used with profit — has been reviewed by McCloskey[59] and by Zeman and Scharmann.[57,58] This is a very effective procedure and when combined with the separating efficiency of gas chromatography it provides one of the most powerful methods of structure determination and requires only a small amount of material.[59,60] Gas chromatography-mass spectrometry (GC-MS) becomes more efficient when linked with a computer. The coupling of mass spectrometry with liquid chromatography[61] should be even more valuable since it can function as a highly sensitive universal detector as well as providing information on structure for identification purposes. The major use of liquid chromatography-mass spectrometry (LC-MS) at present appears to be as an alternative to GC-MS for compounds that

cannot withstand gas chromatographic conditions, but which are sufficiently stable to be volatilisation in the mass spectrometer without excessive decomposition.

Klein[62] reviewed the developments in the use of mass spectrometry as an analytical tool in the field of lipid structural research. Accurate determination of ionic mass is the first step towards a precise description of the various processes by which a molecule fragments. Other especially useful methods for structural elucidation and mechanistic studies involve labelling with stable isotopes and the analysis of metastable transitions.

Electron impact (EI) is the convenient method of ionising samples for mass spectral study. It is a very powerful technique which adequately solves problems in a very large number of cases, but has a number of disadvantages which have resulted in the development of soft ionisation mass spectral methods. Chemical ionisation (CI), field ionisation (FI), and field desorption (FD) are sometimes preferable to EI mass spectrometry as methods for obtaining abundant high-mass ions from lipids.[63] These techniques are commercially available and often called 'soft ionisation' methods because less energy is supplied to the molecules in the mass spectrometric ionisation process and hence the spectra obtained are much simpler than the normal EI spectra since far fewer fragment ions are present. FD often provides

mass spectral information which is unobtainable by other methods, and is the best method for obtaining molecular weight information. Fragment ions are observed in the spectra from all the ionisation methods, which provide structural information complimenting that obtainable from an EI spectrum. Using CI, high-mass ions carrying a large proportion of the total ionisation current can be monitored by selected ion monitoring, resulting in enhanced sensitivity for quantitative studies in some cases.

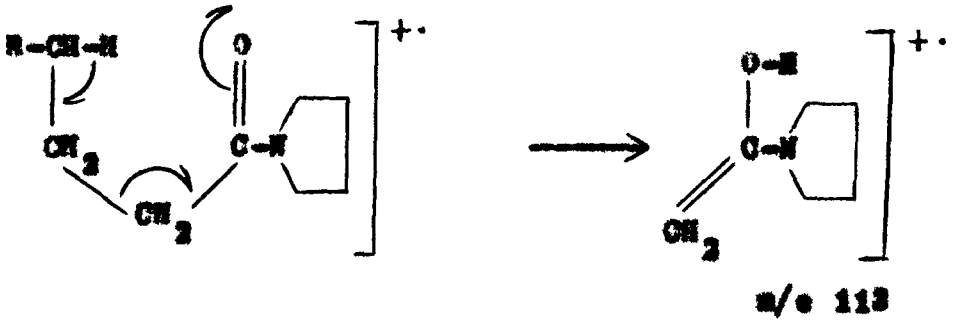
More recently Andersson[64] has claimed that in most cases the structure of an unknown fatty acid may be determined from a study of the mass spectrum of its pyrrolidide. Compared to 'on-site' derivatisation of a functional group in the fatty acid chain, the second approach termed as 'remote' group derivatisation such as pyrrolidides has now assumed importance in MS studies. In such derivatives charge localisation is enhanced at a position remote from the functional group of interest, the latter remaining unaltered. According to Egeles et al.[65] a judicious choice from both 'on-site' and 'remote-group' derivatives will probably remain the most satisfactory approach in the application of MS or GC-MS to the structural elucidation of many organic compounds.

1. Esters of saturated normal chain acids

For methyl esters of saturated fatty acids, the parent ions are observed and two series of fragment ions are formed in

reasonable abundance. The carboxymethoxy ion of the formula $(CH_2)_nCOOCH_3^+$ having m/e=59+14n is derived from the loss of the neutral alkyl fragments. The McLafferty rearrangement (γ - hydrogen migration to oxygen of carbonyl group followed by β- cleavage to the carbonyl group) ion for the methyl ester is $CH_2=C(OH)OCH_3^{+\cdot}$. Cleavage α to ester carbonyl group also occurs in four ways to give ions: (a) $CH_3(CH_2)_n^+$, (b) $CH_3(CH_2)_nC\equiv O^+$, (c) $^+O\equiv C-OCH_3$, and (d) $^+OCH_3$. Fragment ions (a) and (c) are usually of low abundance or absent. Although ions of type (b) (M-31) and (d) (m/e=31) are usually present in low abundance but possess diagnostic value since they are characteristic of the methoxy group in the methyl ester.

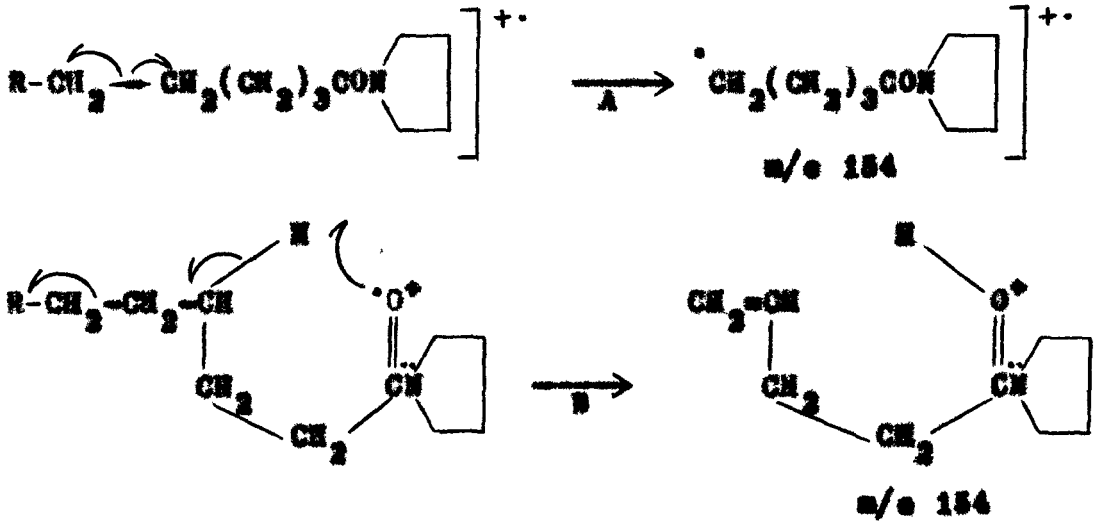
A variety of carboxyl derivatisations have been reported, and of them, the pyrrolidide derivative has been judged to possess the greatest potential for mass spectrometry.[64] Fragmentation of acylpyrrolidides gives a base peak in the spectrum at m/e 113 by the McLafferty rearrangement.



m/e 113

The other mode of fragmentation produces a series of even-massed fragments beginning at m/e 126 incrementing by 14 atomic mass

units (amu) and ending with one carbon less than the molecular ion. These fragments may arise from either homolytic cleavage of C-C bonds (A) or by hydrogen abstraction with cleavage (B). This latter process, an amide-directed fragmentation (ADF), may well be the most favourable reaction pathway.

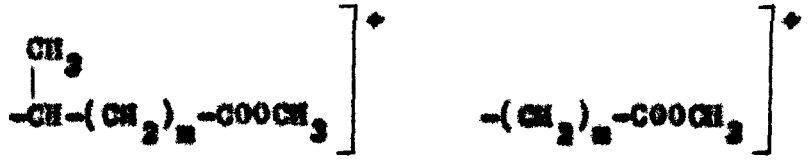


2. Esters containing substituents in the chain

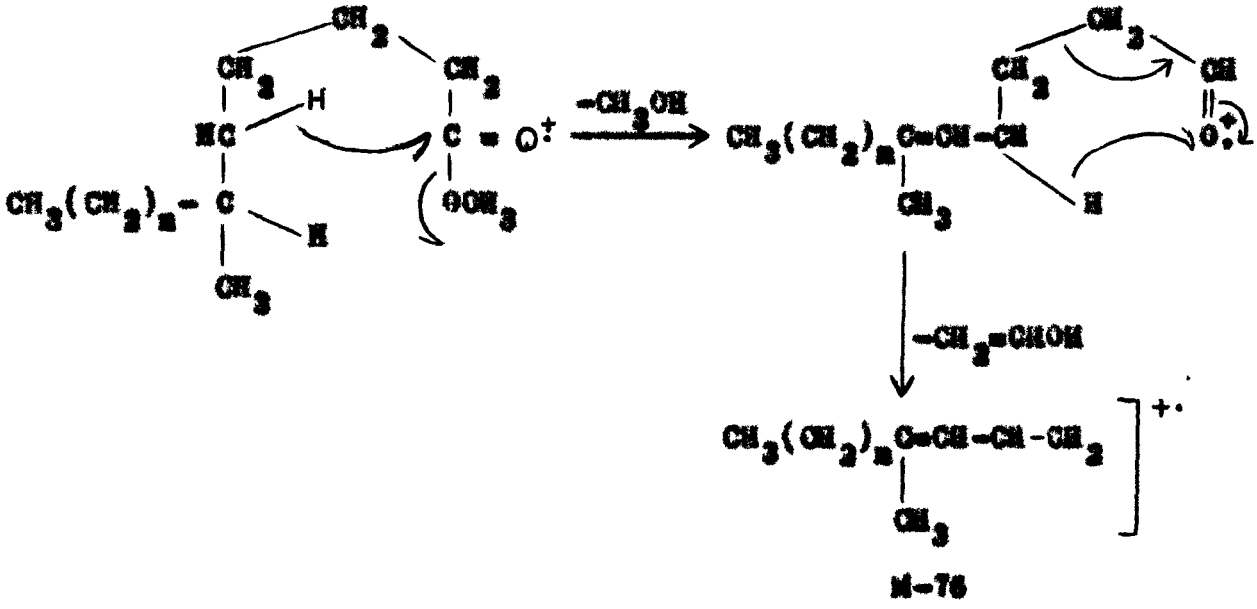
The presence of a functional group in the chain alters the MS fragmentation modes. The position of the functional group can be deduced from the mass spectrum.

Alkyl group. The introduction of an alkyl group in the carbon chain causes changes in the MS fragmentation pattern owing to the facilitation of α-cleavage with respect to the tertiary (3°) carbon atoms. Generally it is possible to deduce the position of a methyl branch by looking for relatively high peaks separated

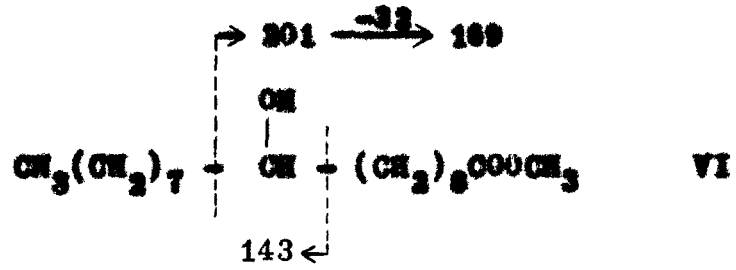
by 28 amu corresponding to the following ions, and, in particular, the absence or low intensity of the intermediate peak separated from its neighbours by 14 amu. The presence of ions formed through loss of methanol (32 amu) or methanol and water (32+18) from the ion that still carries the side chain is a further characteristic feature.



Methyl branching at C-6 leads to an intense peak[55] at M-76.

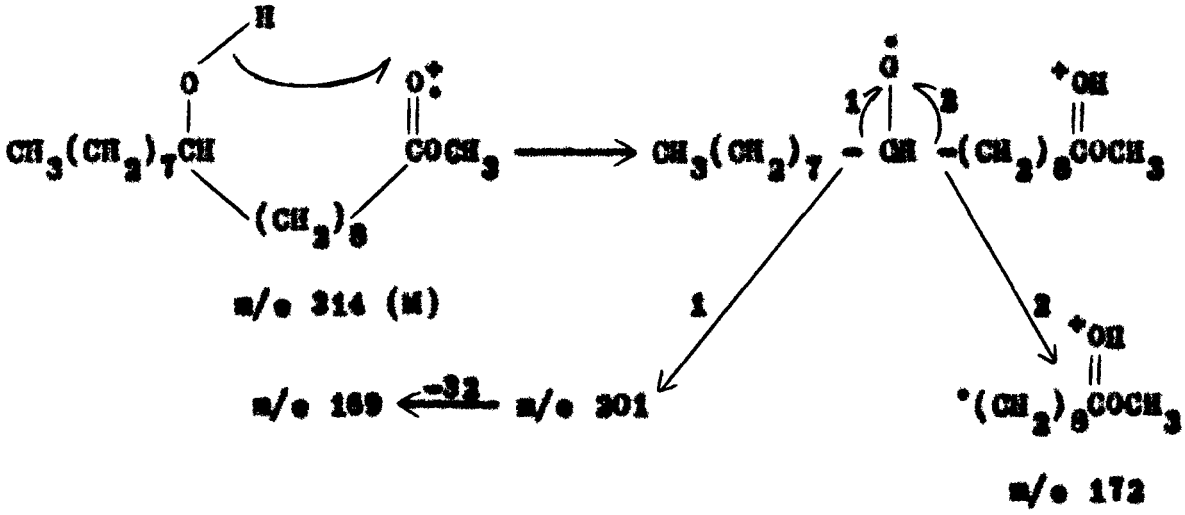


Hydroxyl group. The fragmentation modes caused by hydroxyl group substituent on the hydrocarbon chain can be illustrated by the mass spectrum of 9-hydroxystearate (VI).[66] The ion of the type m/e 201 serves the primary function of marking the position of hydroxyl group substituent in the chain.



VI

The deuterium labelling studies have shown that rearrangement of the hydroxyl hydrogen to the ionised carbomethoxy group occurs at the molecular ion stage. Labelling of the hydroxyl group with deuterium therefore leads to a number of fragment ions containing the label which would not be expected on the basis of simple cleavages.



For structure elucidation of hydroxy esters by MS studies, the best results were obtained by converting them to trimethylsilyloxy (OTMS) derivatives.[67] Such derivatives are used because of their great volatility and ease of preparation. The McLafferty rearrangement product, m/e 132 and members of the

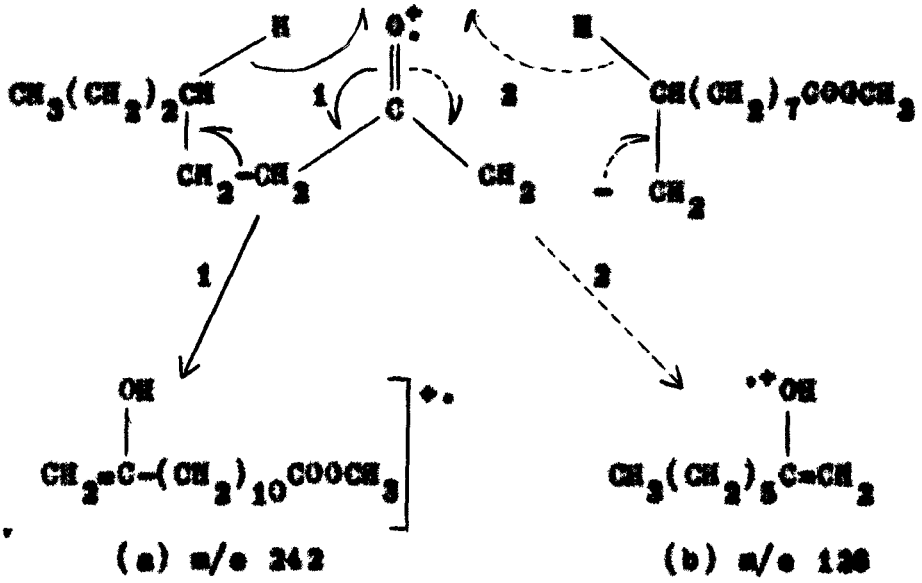
$TMSOCO(CH_2)_n^+$ series are found 58 amu higher than their methyl ester counterparts. The acylium ion M-OTMS is not present. The most abundance ions in the spectrum are due to the trimethylsilyl ion, m/e 73 and the rearranged ion m/e 75, both of which are found in virtually all mass spectra of TMS derivatives.

$$-CH(OH)- \longrightarrow -CH[OSi(CH_3)_3]-$$

$$^+Si(CH_3)_3 \qquad H\overset{+}{O}{=}Si(CH_3)_2$$

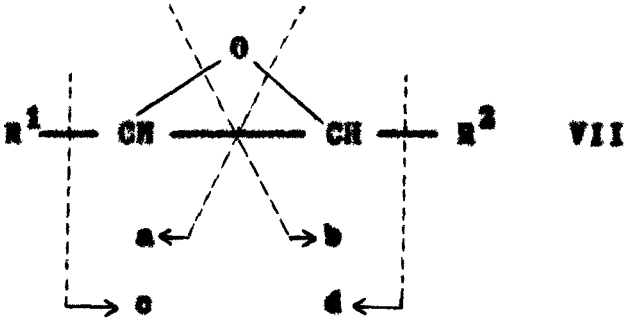
m/e 73   m/e 75

<u>Oxo group</u>. The position of a keto oxygen atom generally can be deduced easily from the mass spectrum,[66] considering the simple α-cleavage as well as β-cleavage with rearrangement. In keto esters, an additional characteristic mode of fragmentation arises from McLafferty rearrangement of either γ-hydrogen to the carbonyl function in the chain, giving ions (a) and (b) by routes 1 or 2, respectively. Recognition of (a) and (b) is facilitated by their even-numbered (i.e. odd-electron) character.



(a) m/e 242 (b) m/e 126

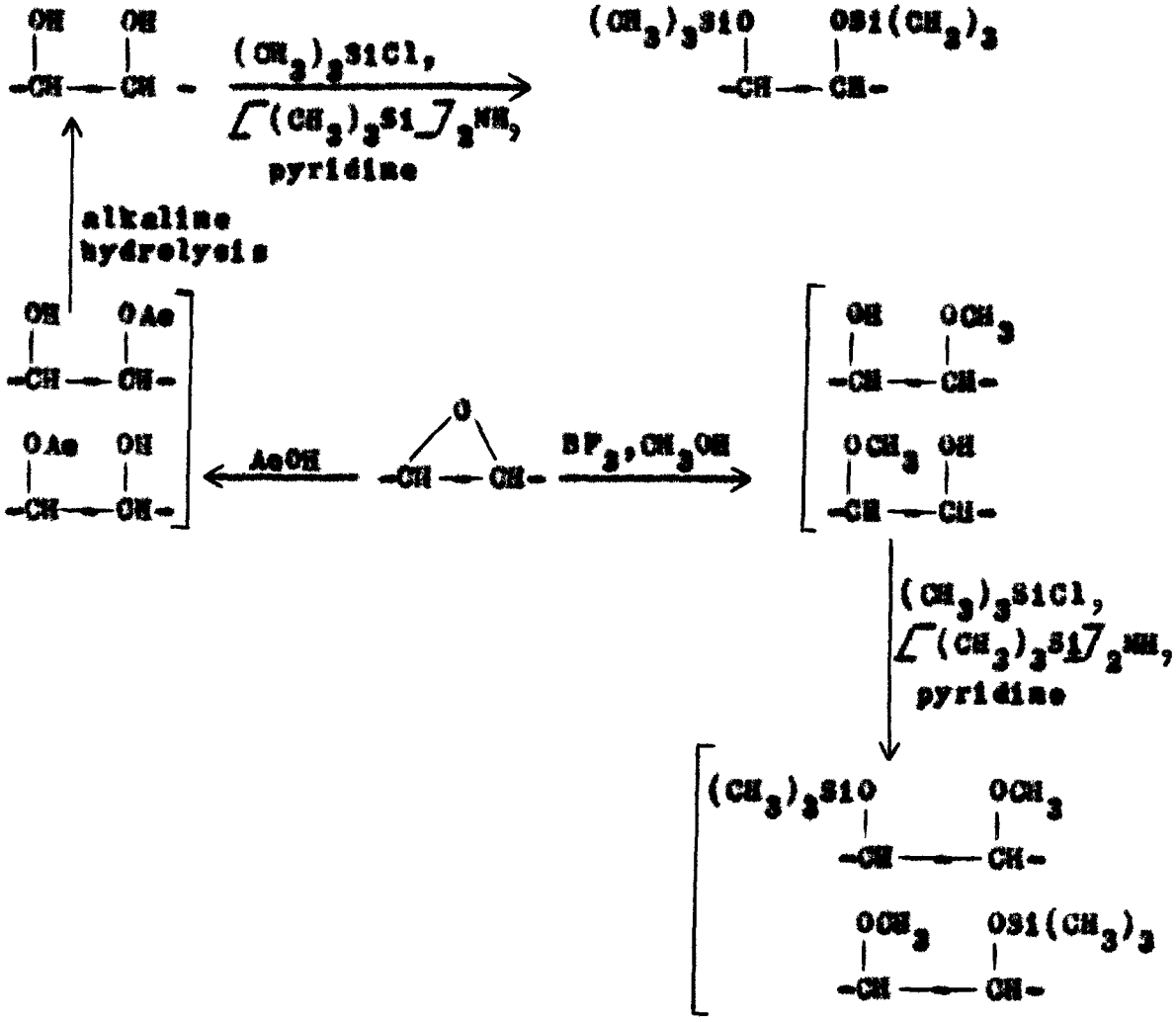
Epoxy group. Saturated epoxides give structurally informative mass spectra based on the major fragmentations shown in VII.[68,69]



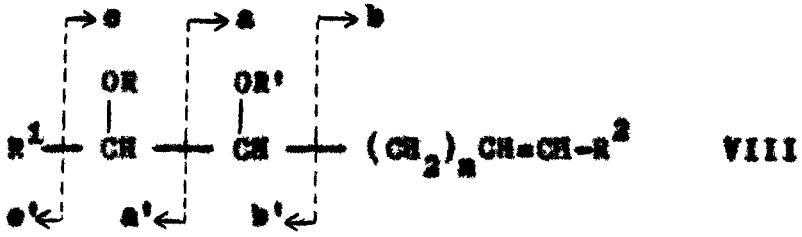
VII

Unsaturated epoxides are better identified by mass spectrometry after conversion to dihydroxy or hydroxymethoxy derivatives with the reagents shown in Scheme 2 and subsequent conversion to bis(trimethylsilyloxy) or methoxy trimethylsilyloxy ethers.

Scheme 2



These derivatives undergo fragmentation between the two ether-bearing carbon atoms or on either side of these[70,71] as shown in VIII.

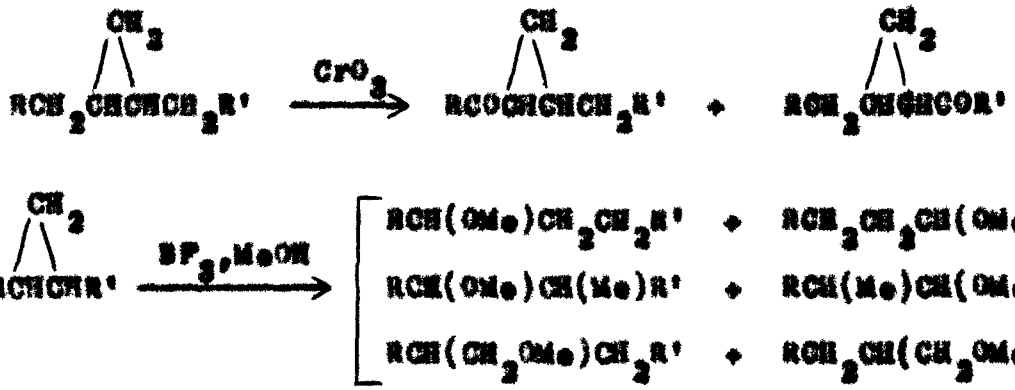


VIII

Minnikin and Patel[73] have studied t-butyldimethylsilyloxy ether derivatives of iodine-catalysed solvolysis products of long-chain epoxides. The spectra of these derivatives contained intense fragments allowing the molecular weights and the positions of the ether functions to be easily determined. Very recently Egeles et al.[88] have carried out detailed MS study of pyrrolides of a number of fatty acids containing oxygen functions (ether linkage, epoxide ring, and hydroxyl group). Their results indicate that the presence of the oxygen atom has a profound effect on the normal sequential mode of pyrrolidide fragmentation.

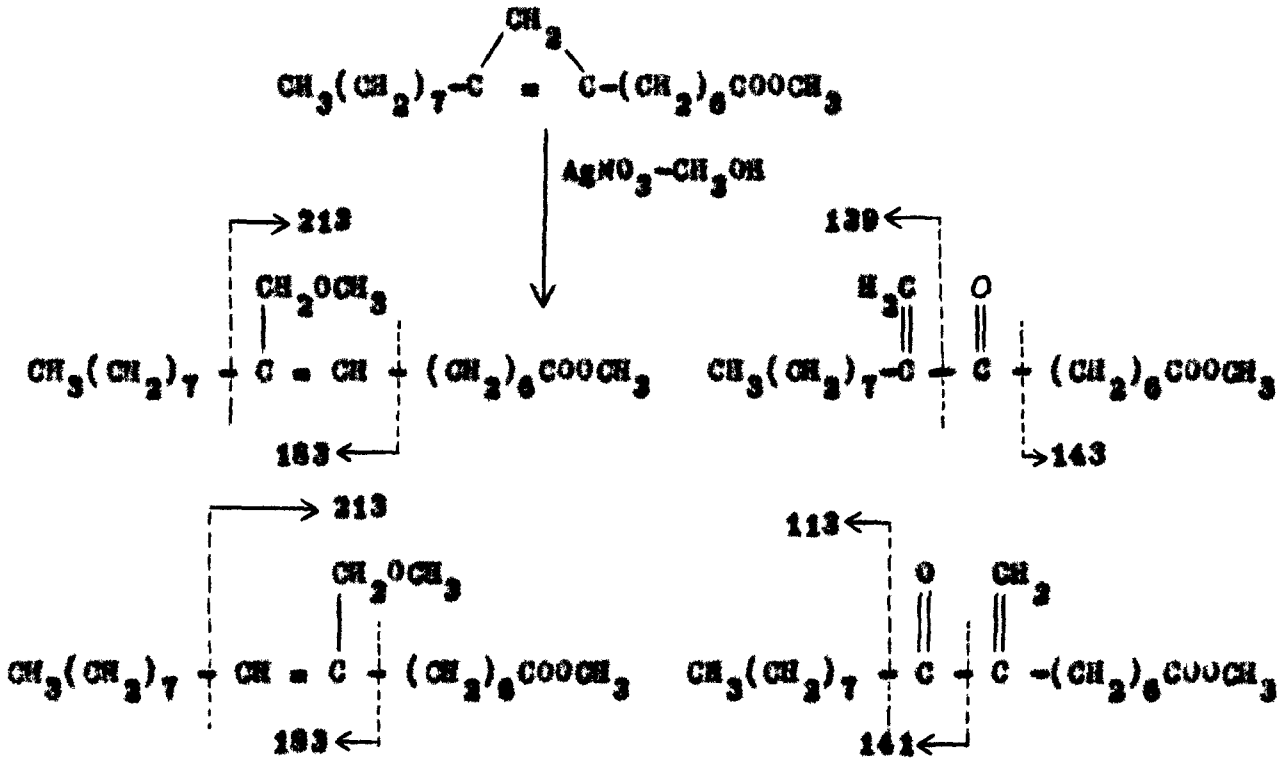
Cyclopropane and cyclopropene groups. Cyclopropane compounds are generally unstable under electron bombardment and give spectra of little diagnostic value. The cyclopropane system may be fixed by some chemical reactions[73] leading to a product, or more usually a mixture of products, which are identified by mass spectrometry. These reactions include hydrogenolysis, oxidation, and reaction with methanolic BF_3.

$$RCH\overset{CH_2}{\diagup\diagdown}CHR' \xrightarrow[AcOH]{H_2,Pt} RCH\overset{CH_3}{|}CH_2R' + RCH_2\overset{CH_3}{|}CHR' + RCH_2CH_2CH_2R'$$



Pawlowski *et al.*[74] have reported the mass spectra of cyclopropene fatty acid (CPFA) esters, methyl malvalate and methyl sterculate. Kisele *et al.*[75] and very recently Ahmad *et al.*[76] have carried out GC-MS study of silver nitrate-methanol treated methyl esters of CPFA. The method of derivatisation and MS fragmentation pattern for malvalic acid are shown in Scheme 3.

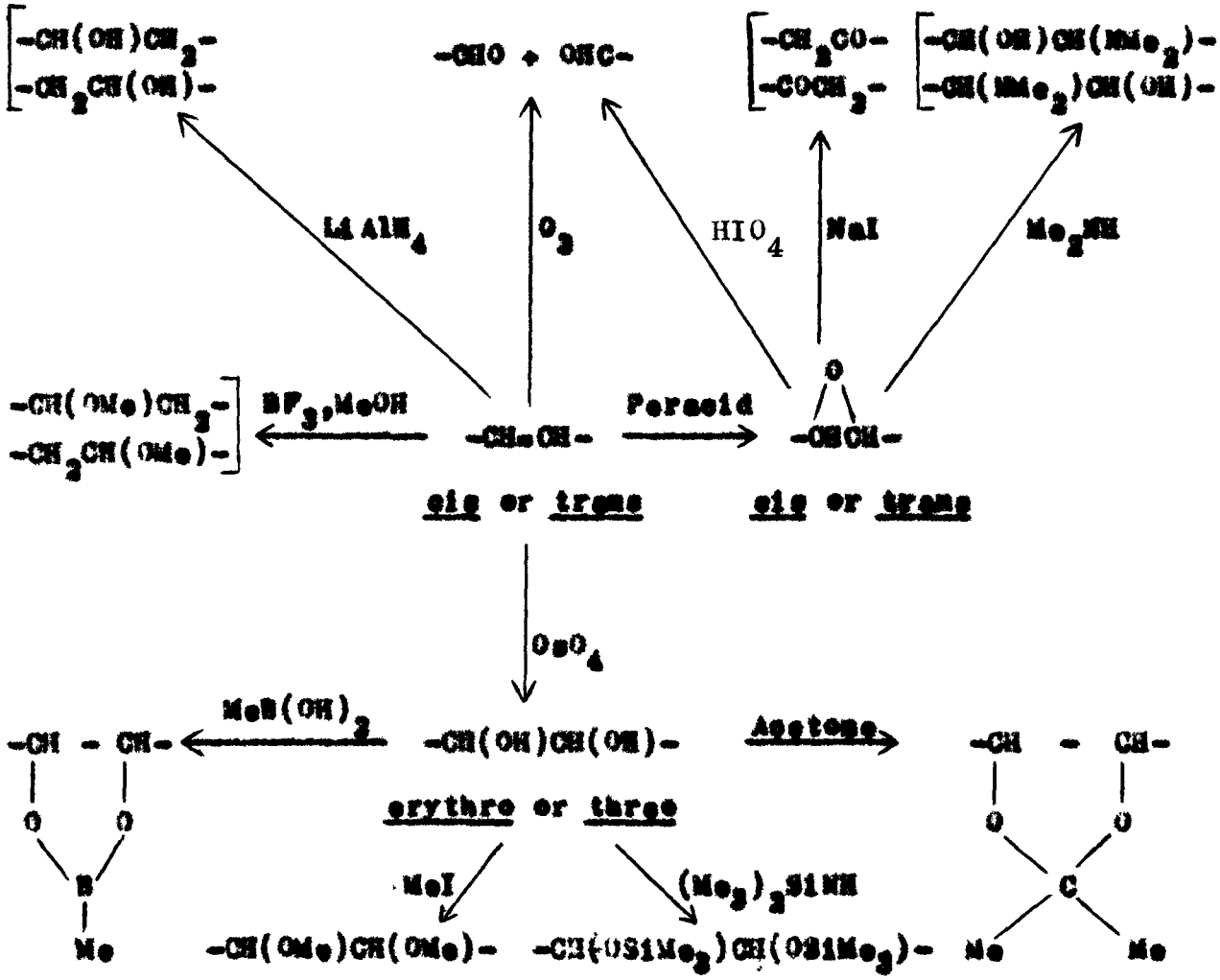
Scheme 3



Mass spectrometry of pyrrolidides has also been investigated as a possible procedure for the location of cyclopropane[73] and cyclopentane[77] rings.

3. Esters containing unsaturation

Double bond. Structure determination involves, more often, the determination of double bond position and it is unfortunate that mass spectrometry does not provide an immediate solution to that problem. The double bond system is labile on electron bombardment and isomeric esters give almost indistinguishable mass spectra of little diagnostic value. Since 1960 various procedures for overcoming this difficulty have been proposed. Each procedure involves a chemical modification of the alkenoate to "fix" the double bond. Some reactions give mixed products and this complicates the interpretation a little. This is not too serious with monoenoates but the situation may become confused with polyunsaturated esters and although mass spectrometry has been applied to such systems no single widely applied procedure has been evolved. The following reactions have been reported for the mass spectrometric study of alkenoates.[73]



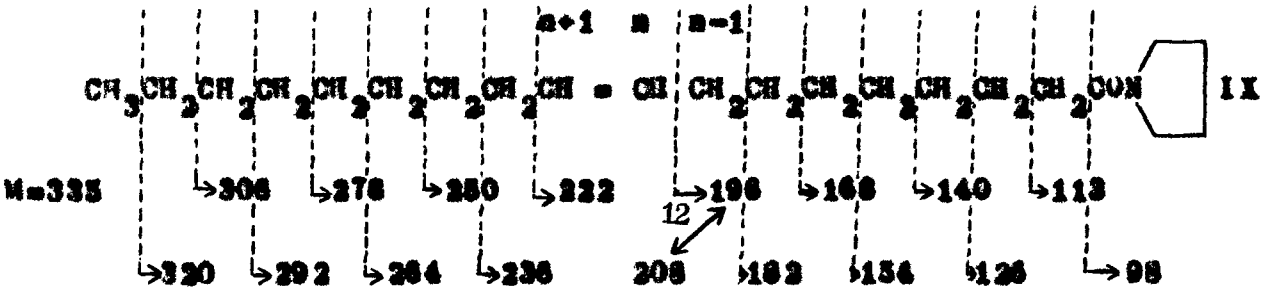
Oxymercuration-demercuration of unsaturated esters is also a useful procedure[73,78] for the resolution of complex mixtures. The ready formation of cyclic ethers on oxymercuration-demercuration was developed by Gunstone and Inglis[79] as a method for the location of unsaturation in $\Delta 3$ (*trans*) and *cis* and *trans* $\Delta 4$ and $\Delta 5$ positions.

The problem of double bond migration during electron impact mass spectrometry of unsaturated esters may be avoided by analysis of amide derivatives particularly pyrrolidides. Anderson and Holman[80] studied the mass spectra of a range of monounsaturated

fatty acid pyrrolidides and from their experience with $\Delta 5 - \Delta 15$ octadecenoates formulated the following rule:

"If an interval of 12 amu, instead of the regular 14, is observed between the most intense peaks of clusters of fragments containing n and n-1 carbon atoms of the acid moiety, a double bond occurs between carbon n and n+1 in the molecule".

This rule is illustrated by reference to the mass spectrum of the pyrrolidide of oleic acid (IX).[80]



Triple Bond. The basic mass spectral pattern of acetylenic fatty acids is one of cleavage with McLafferty rearrangement either of the triple bond or of the isomeric allenes formed by rearrangement.[81] Therefore, electron bombardment of each octadecynoate should produce four characteristic ions A, B, C and D.

$$CH_3(CH_2)_m-C \equiv C-(CH_2)_nCOOCH_3$$

$CH_3(CH_2)_m-\overset{+}{C}=C=CH_2$ A

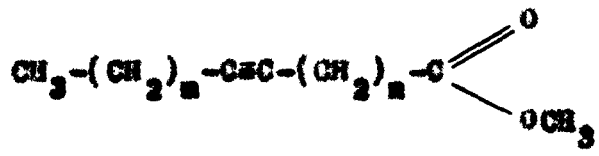
$CH_3(CH_2)_m-CH=\overset{+}{C}-CH=CH_2$ B

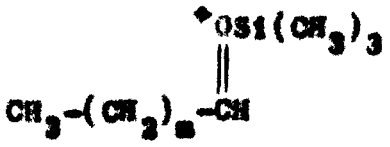
$CH_2=C=\overset{+}{C}-(CH_2)_nCOOCH_3$ C

$CH_2=CH-\overset{+}{C}=CH-(CH_2)_nCOOCH_3$ D

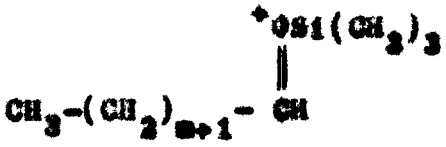
Ions containing the terminal part of the molecule (A and B) are the most abundant when the triple bond is close to this part of the molecule. Type C and C-32 are the most intense of the characteristic ions when the triple bond is near the ester function. In fact, when the triple bond is in C-4 to C-6 positions, ions A and B are not detected, and the spectra of the C-14 and C-15 isomers do not show ions of type C and D.

Oxymercuration of acetylenic esters gives isomeric hydroxy esters when demercurated with $NaBH_4$. The hydroxy esters after silylation, give diagnostic mass spectra indicative of the triple bond location. Analogous to oxymercuration of double bonds, when the triple bond is close to the ester group, the hydroxyl group forms only on the carbon atom farther from this functional group. Therefore, only 3-hydroxystearate is formed from 2-octadecynoate and 5-hydroxystearate from 4-octadecynoate.[81] Each derivatised octadecynoate produces up to six characteristic ions E, F, G, H, I, and J. The ions E and F predominate when the OTMS group is close to the ω-methyl group, and the rest are dominant when the functional group is nearer to the ester function. Ions 32 amu (CH_3OH) less than fragments G and H are also found.

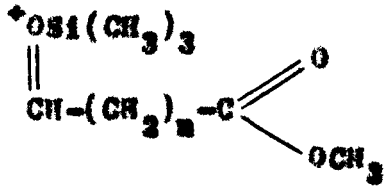




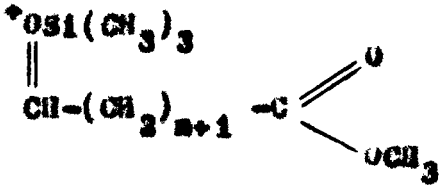
E



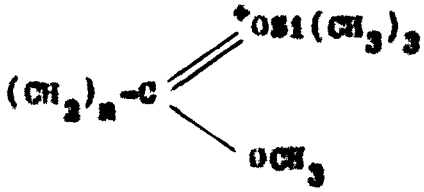
F



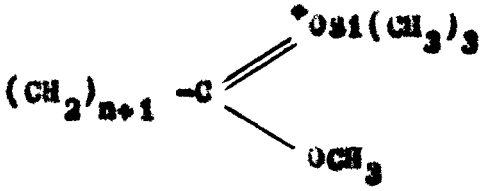
G



H

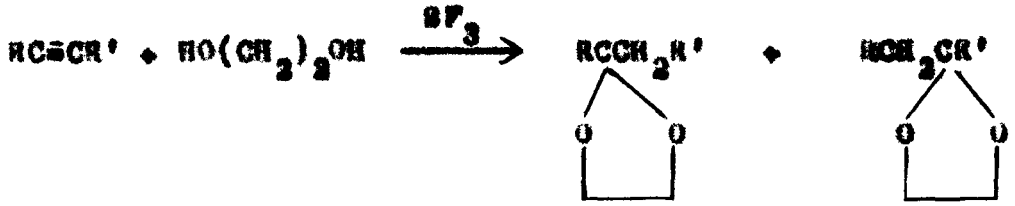


I



J

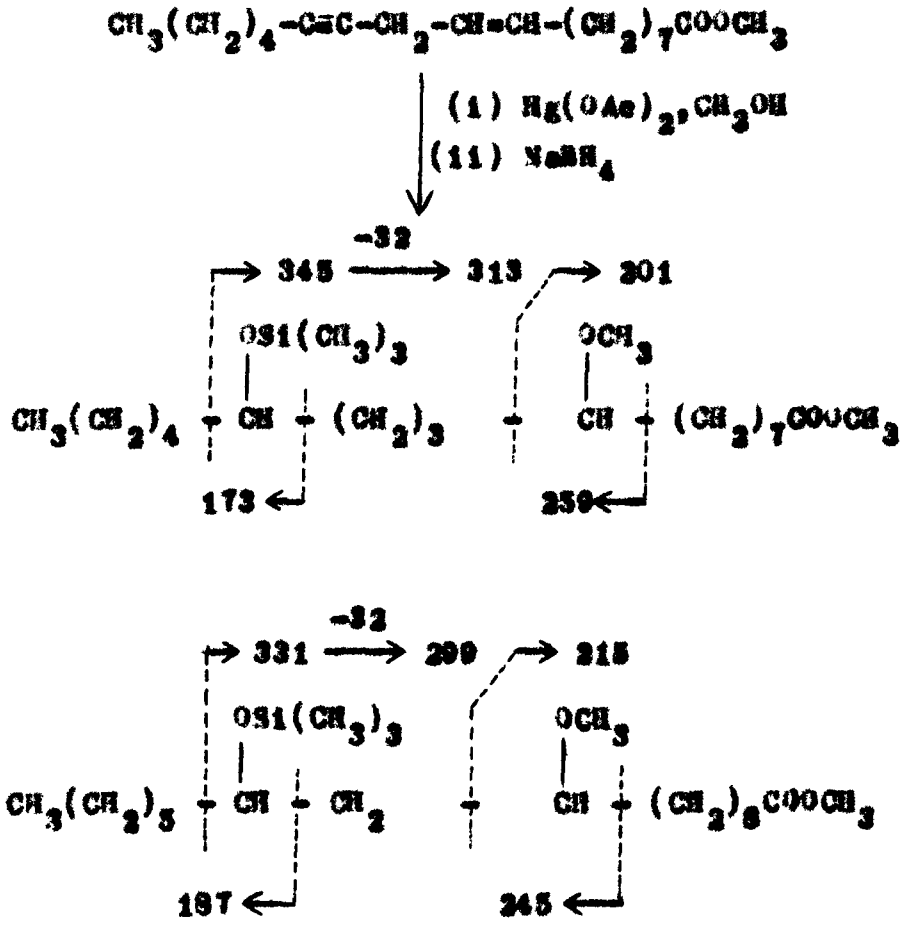
Acetylenic groups can also be placed directly after conversion to ethylene ketals:[32]



The mass spectra of acetylenic fatty acids are complex and the complexity is greater when double bonds accompany the triple bonds. By applying oxymercuration procedures[41] to monoacetylenic and enynoic esters, both double and triple bonds

form derivatives which can be used to give definite mass spectra that locate and differentiate the two types of unsaturation. Oxymercuration in methanol and reduction with $NaBH_4$ result in two isomeric methoxy esters for each double bond and two isomeric hydroxy esters for each triple bond. For example the mass spectrum of methyl eropenynate[91] clearly locates the original triple bond (m/e 173 and 187) and the double bond (m/e 201 and 215) (Scheme 4).

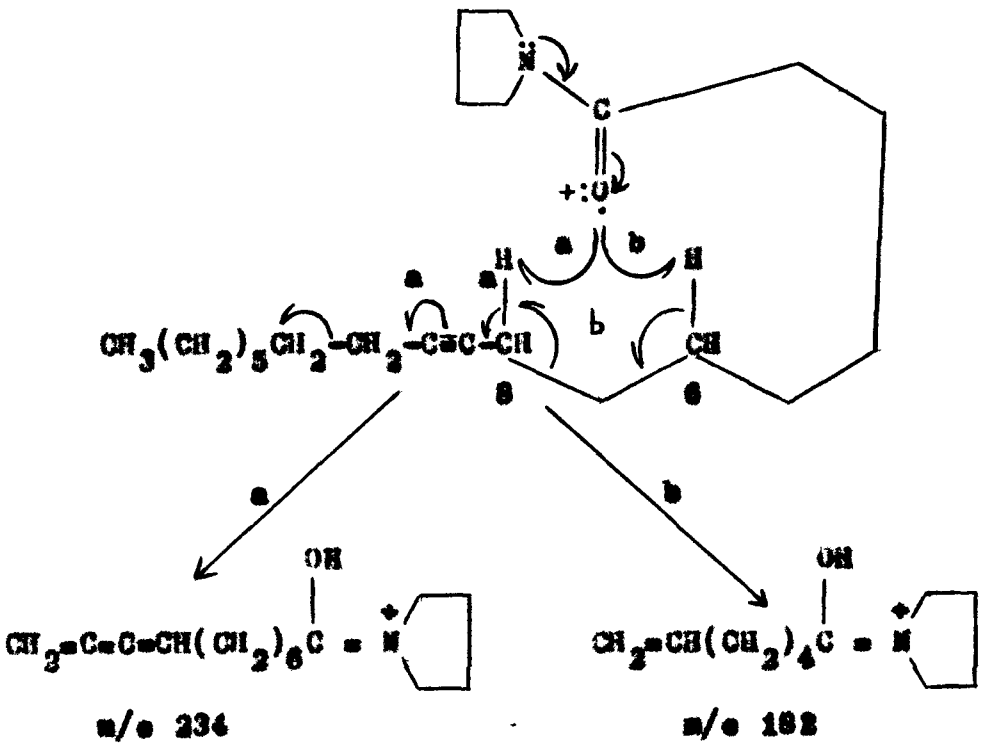
Scheme 4



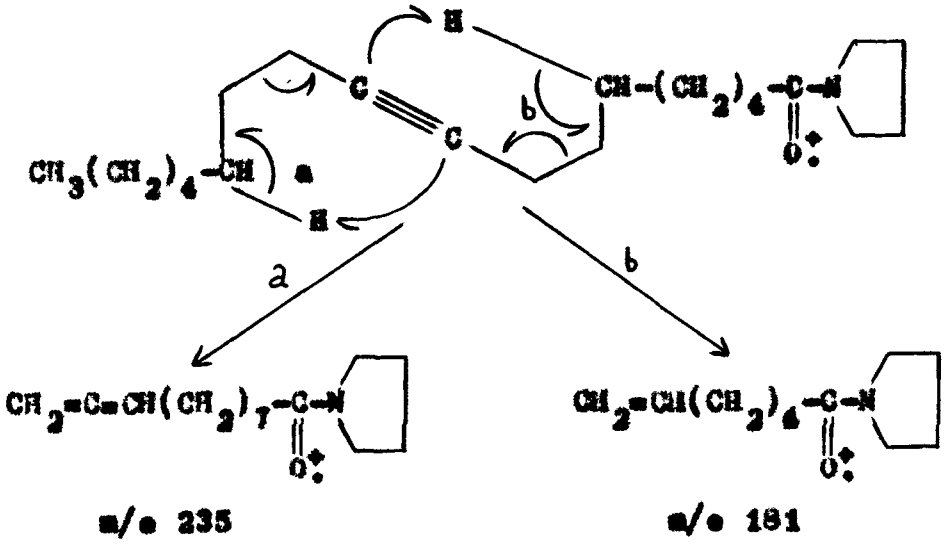
Silylation of esters that have a conjugated diene or enyne system adjacent to hydroxyl group gives derivatives amenable to GC-MS.[70] In these esters, large peaks are observed that arise from α-cleavage at the OTMS group and at the other end of the olefinic system. No fragmentation between the OTMS group and the sites of unsaturation occurs.

Very recently Valicenti *et al.*[83] have reported the MS analysis of pyrrolidide derivatives of a series of isomeric octadecynoic acids. An interval of 10 amu (rather than the usual 14) between fragments corresponding to C_{n-2} and C_{n-1} of the acyl moiety indicates a triple bond at C_n. Confirmation of triple bond location is provided by intense peaks at C_{n-2} and C_{n+2}. For the location of triple bond, amide-directed fragmentation (ADF) is the more significant mechanism. As the acetylenic unsaturation approaches the carboxylic group, substituent-directed fragmentation (SDF) becomes more evident and odd-mass ions are produced. Scheme 5 illustrates the two competitive processes which may produce large C_7 and C_{11} fragments in the mass spectrum of *N*-octadec-9-ynoylpyrrolidine. The large, even-mass peaks, m/e 192 and 234, are only possible with the ADF mechanism; SDF derived peaks (m/e 191 and 235) are of minor importance in the spectrum.

Schema 5



ADF



SDF

DISCUSSION

Besides the tremendous development in the field of oils and fats, the need of fatty chemicals as raw-materials for use in oil-based industries was realised in recent years. A number of sites are available in the fatty acid chain for modifications to provide new routes for the synthesis of various derivatives.

Acetylenic unsaturation in naturally occurring fatty acids is not as common as olefinic unsaturation. The acetylenic intermediates have versatile use in organic synthesis. That is why the acetylenic linkage has drawn considerable attention to a fat chemist. Since acetylenic fatty acids are not commercially available, attempt has been made to synthesise them and study their chemistry. The model acids selected for the present investigation were 9- and 10-undecynoic acids. They were chosen as prototypes of long-chain acetylenic acids in general, since the symmetry in former, terminal linkage in later, and lack of interferring groups in both instances were expected to simplify product isolation and identification. The work described in this section deals with the preparation of the aforesaid undecynoic acids and their reactions with peroxyacid, hypohalous acid, and iodine azide.

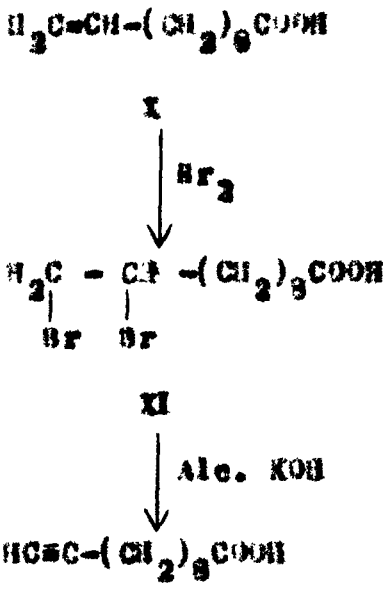
1. Preparation of 10-undecynoic acid (XII)

For the preparation of 10-undecynoic acid the method of Kamm et al.,[84] which involves bromination of olefinic fatty

acid followed by dehydrobromination of resulting dibromo acid by alcoholic potassium hydroxide (Scheme 6), was adopted in the present work.

10-Undecenoic acid (X) on treatment with bromine in carbon tetrachloride gave almost a theoretical yield of 10,11-dibromoundecanoic acid (XI). The resulting dibromide (XI) was then subjected to dehydrobromination by refluxing with potassium hydroxide and ethanol. After evaporation of alcohol, the product was worked-up in usual manner. The crude material on crystallisation from petrolium ether at low temperature furnished a crystalline product (XII) (TLC homogenous,

Scheme 6



yield ~55%) melting at 42°C. The elemental analysis of this compound corresponded to the molecular formula $C_{11}H_{18}O_2$.

The identity of XII was further established by the spectral data of its methyl ester (XIIA). The IR spectrum gave bands at 3270s (C-H str), 2110w (C≡C str), and 1740s cm^{-1} (C=O str). The NMR spectrum gave the following absorptions with proton integration, signal multiplicity, and probable assignments in parentheses: δ 1.32 (12H, broad, s, $-CH_2-$), 1.90 (1H, t like, $HC\equiv C-$, J=2 Hz), 1.98-2.40 (4H, overlapping signals, $-C\equiv C-CH_2-$ and $-CH_2-COO$), and 3.60 (3H, S, $-COOCH_3$). The small coupling constant (J=2 Hz) of the signal for terminal acetylenic proton suggested a long range coupling between C-9 methylene and C-11 acetylenic protons.

The mass spectrometry was more informative regarding the structure elucidation of XIIA. The genesis of fragment ions discussed can be rationalised according to the schemes given in the thesis. The mechanistic schemes suggested are tentative in the absence of spectra of appropriate deuterated analogues of the compounds under discussion.

The mass spectrum of XIIA (Figure 5) has the same fragmentation pattern as reported by Kleiman et al.[61] for a series of acetylenic esters. The molecular ion peak (M^+) was observed at m/e 196 ($C_{12}H_{20}O_2$) along with associated peaks at m/e 165 (M-31, loss of OCH_3), 164 (M-32, loss of CH_3OH), 137 (M-59, loss of $COOCH_3$), 122 (M-74, fragment left after the loss of ion formed by McLafferty rearrangement), and 74 (arised by

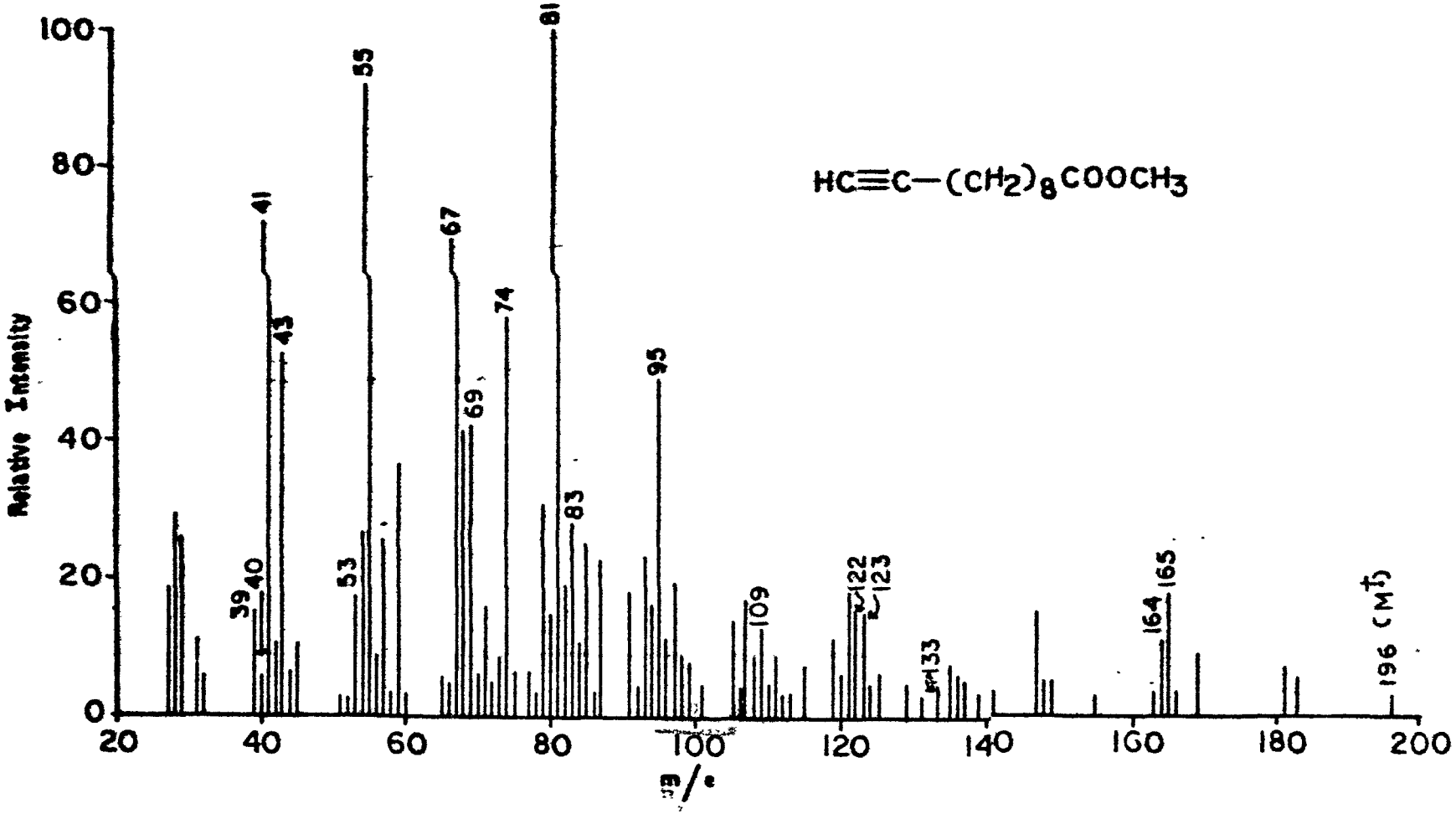


Fig 5. Mass Spectrum of Methyl 10-undecyneate (XIIA)

McLafferty rearrangement). Other characteristic peaks at m/e 123, 109, 95, 83, 81 (base peak), 69, 67, 55, 53, 43, 41, 40, and 39 are considered to arise according to the following Schemes:

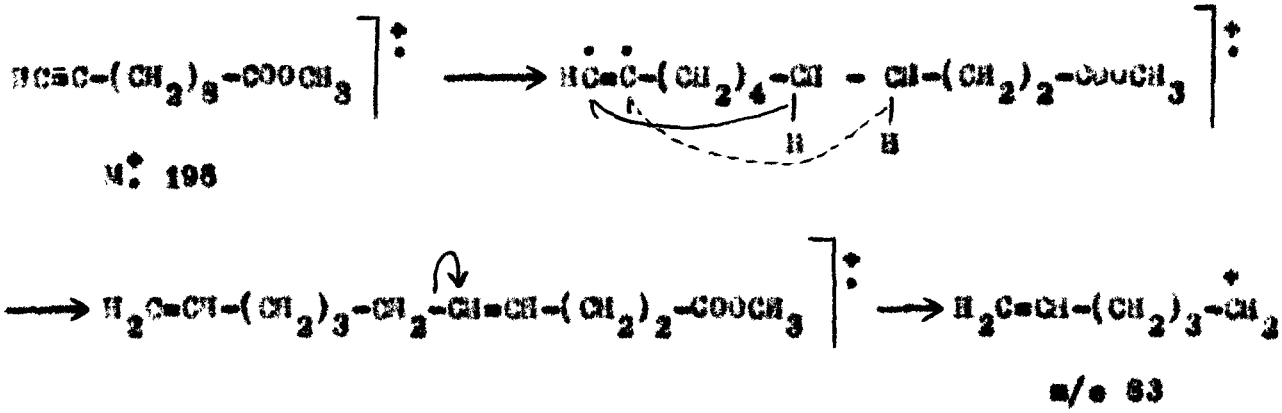
m/e 123, 109 and 95

The fragment ions m/e 123, 109 and 95 obviously result by subsequent losses of CH_2 (14 amu) from the ion m/e 137 (Δ-$COOCH_3$).

m/e 83

This fragment ion can be shown to arise by the cleavage after rearrangements shown in Scheme 7.

Scheme 7



m/e 83

m/e 81, 69 and 67

The fragment ions m/e 81, 69 and 67 result by the loss of CH_2 from the ions m/e 95, 83, and 81, respectively. The ion peak

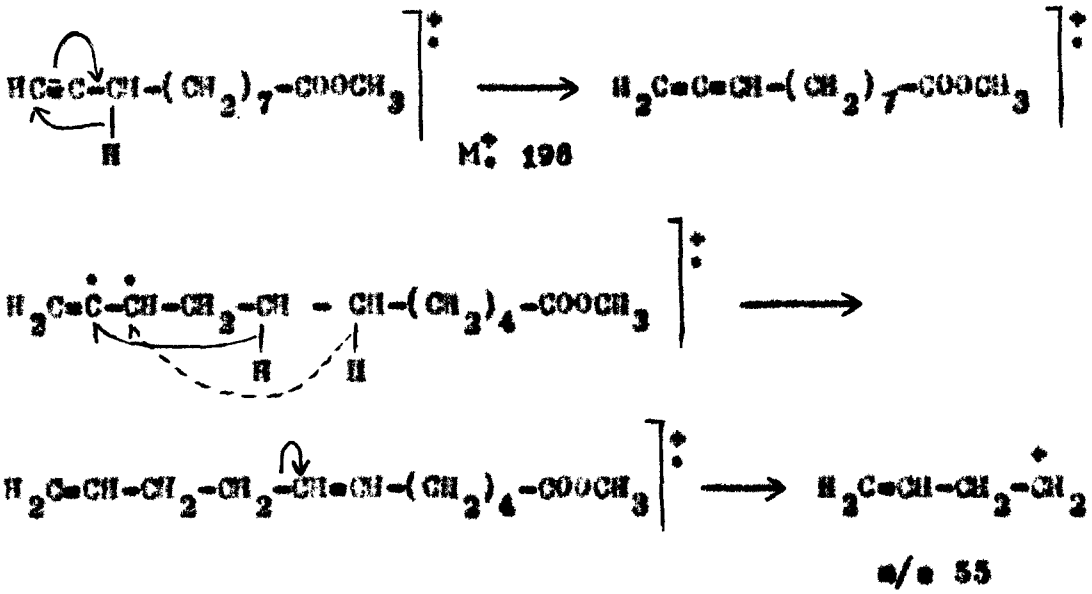
at m/e 91 constitutes the base peak of the spectrum. The cleavage resulting the ion m/e 91 is shown in Scheme 8.

Scheme 8

m/e 55

The ion peak at m/e 55 is quite prominent and the corresponding ion is derived from the rearranged allenic acid according to Scheme 9.

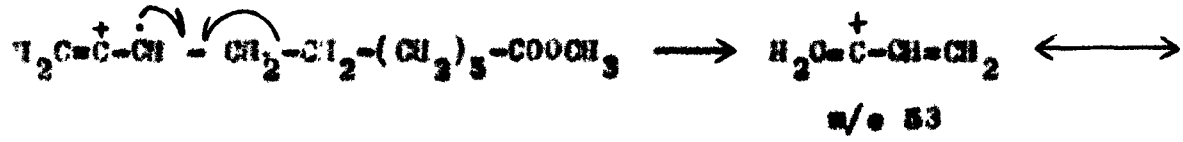
Scheme 9



m/e 53

The fragment ion m/e 53 corresponds to the loss of CH_2 from the ion m/e 67. This fragment ion can also be originated following the way depicted in Scheme 10.

Scheme 10

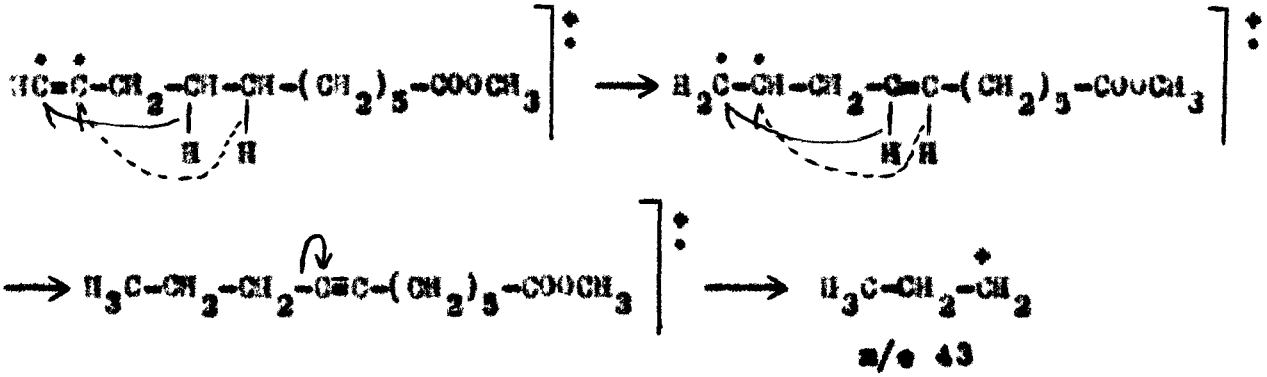


m/e 53

m/e 43

This fragment ion could possibly be obtained from the molecular ion according to the mechanism shown in Scheme 11.

Scheme 11

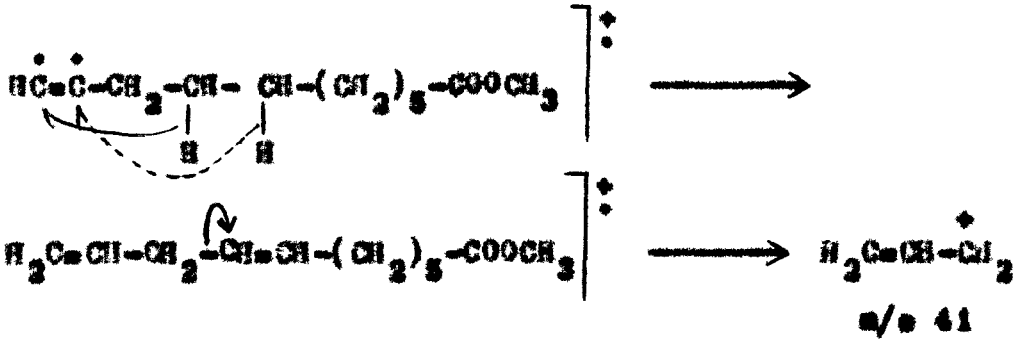


m/e 43

m/e 41

The fragment ion m/e 41 is obtained by the loss of CH_2 from the ion m/e 55. This ion can also be shown to arise from the molecular ion according to Scheme 12.

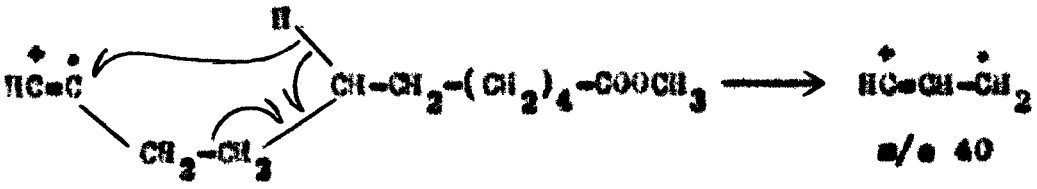
Scheme 12



m/e 40

The fragment ion m/e 40 is formed from the molecular ion by γ-hydrogen migration to the triple bond followed by β-cleavage (McLafferty rearrangement with respect to triple bond). The mechanism is shown in Scheme 13.

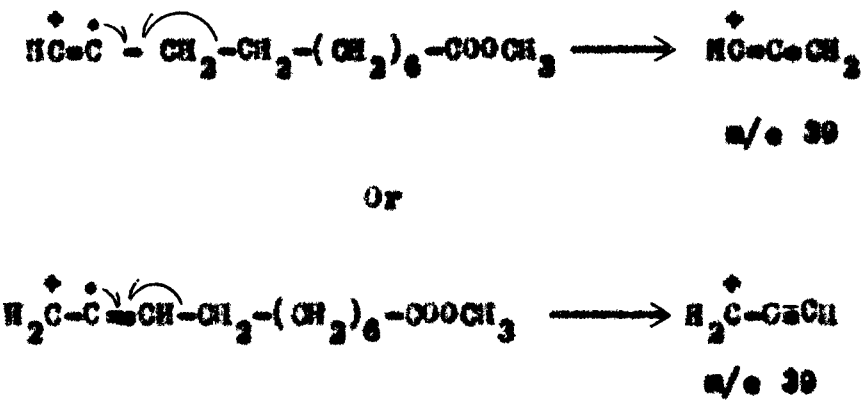
Scheme 13



m/e 39

The ion m/e 39 results by the loss of CH_3 from the ion m/e 53. This is an important characteristic peak for the location of triple bond between C-10 and C-11. This fragment ion can also be obtained from acetylenic as well as rearranged allenic acid (Scheme - 14).

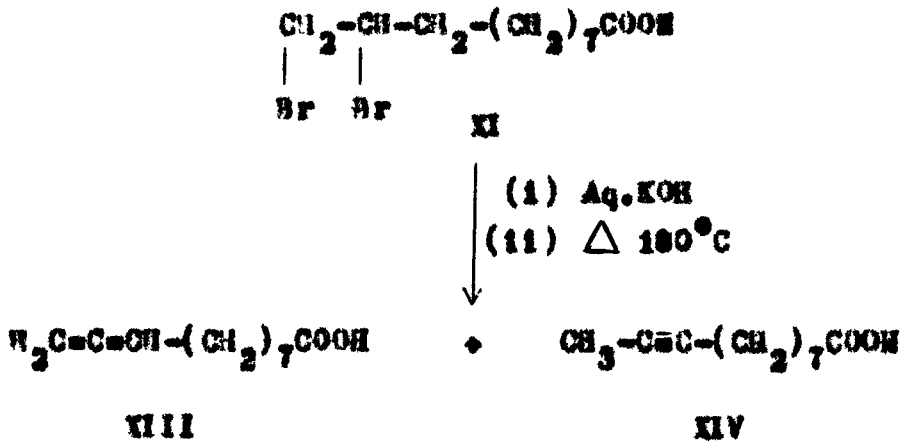
Scheme 14



2. Preparation of 9-undecynoic acid (XIV)

The method of Ames and Bowman[85] involving dehydrobromination of dibromo derivative of olefinic fatty acid by aqueous potassium hydroxide was followed for the synthesis of 9-undecynoic acid (Scheme 15).

Scheme 15



10,11-Dibromoundecanoic acid (XI) obtained by bromination of 10-undecenoic acid (X) was subjected to dehydrobromination by heating with concentrated aqueous potassium hydroxide at 180°C. After usual work-up of the reaction mixture, the product was obtained as non-crystalline oil. The oily product, revealing two spots in TLC, was applied to column chromatography. Second crystalline fraction obtained from the column was characterised as 9-undecynoic acid (XIV, m.p. 59°C, yield ~45%). The pure acid gave single spot on TLC plate. The microanalysis of the compound revealed the molecular formula $C_{11}H_{18}O_2$. The IR spectrum of the methyl ester (XIVA) showed characteristic bands at 2210w (C≡C str) and 1740s cm^{-1} (C=O str). The absence of the strong band in the region 3305-3270 cm^{-1} (characteristic of mono-substituted acetylenes) and the presence of the weak band at 2210 cm^{-1} indicated the compound as 1,2-disubstituted acetylene.

The penultimate acetylenic structure of XIVA was further supported by the signals in NMR spectrum. The signals appeared at δ 1.35 (10H, broad S, $-CH_2-$), 1.74 (3H, t like, $CH_3-C\equiv C-$, J=2 Hz), 2.0-2.50 (4H, overlapping signals, $-C\equiv C-CH_2-$ and $-CH_2-COO$), and 3.65 (3H, s, $-COOCH_3$). The small coupling constant of the signal for terminal methyl protons suggested that there is a long range coupling between C-8 methylene and C-11 methyl protons. No molecular ion peak at m/e 196 ($C_{12}H_{20}O_2$) was observed in mass spectrum of XIVA (Figure 6). The highest mass peak was observed at m/e 197 (M+1) along with associated

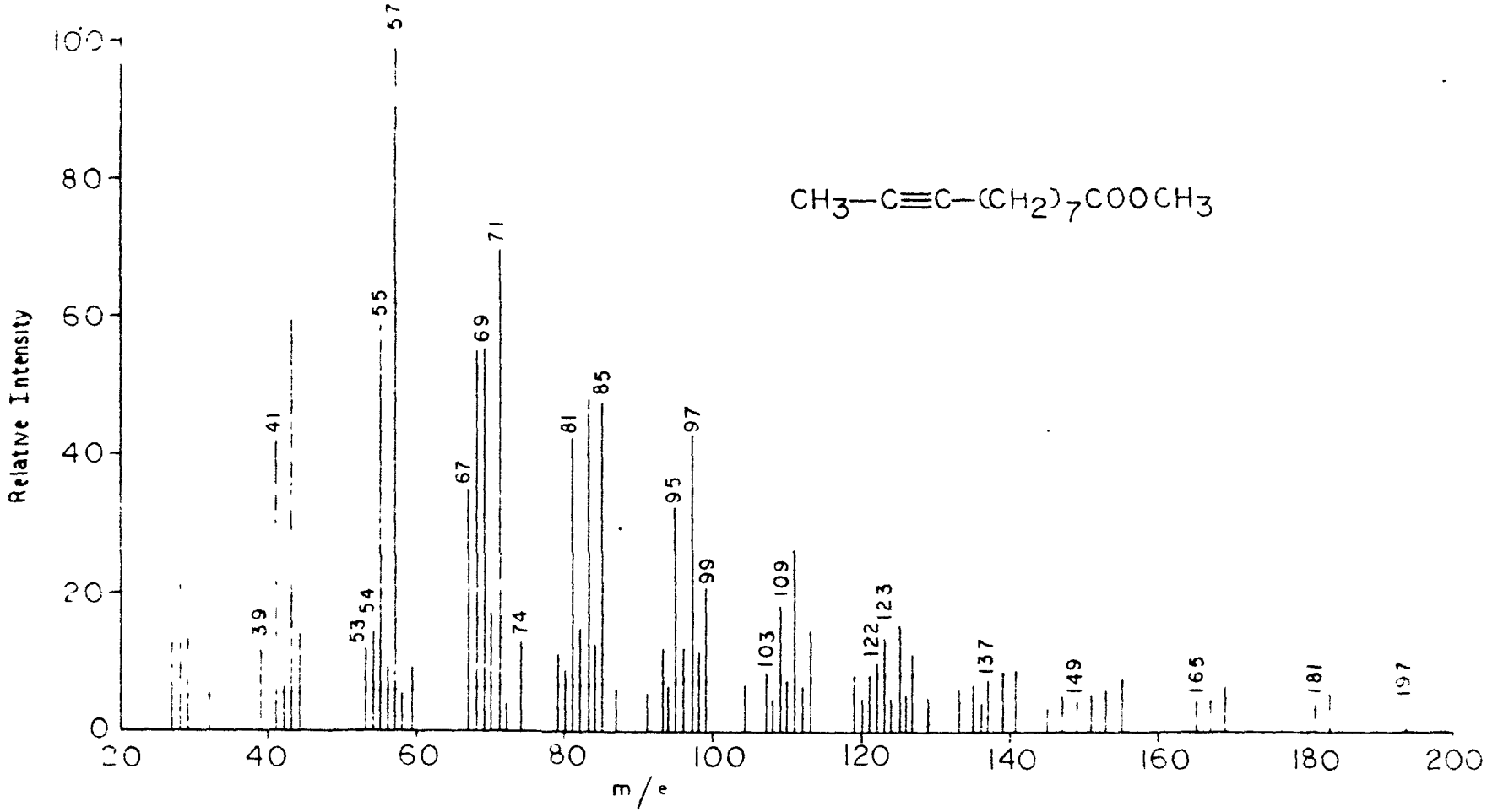


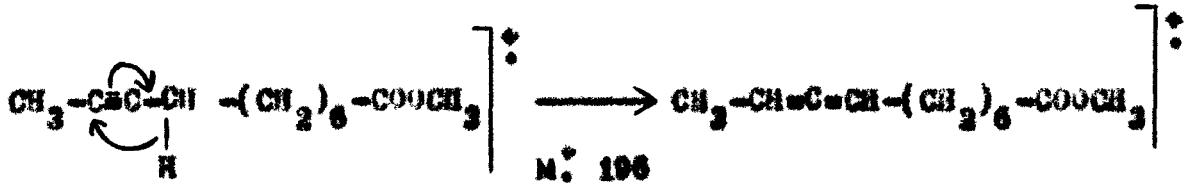
Fig. 6. Mass Spectrum of Methyl 9-undecynoate (XIVA)

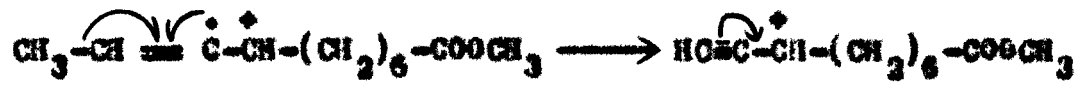
peaks at m/e 165, 137, 123, 122, 109, 95, 91, 67, 53, and 39 in common as discussed in the case of methyl 10-undecynoate (XIIA). The genesis of other significant peaks at m/e 181, 149, 107, 99, 97, 85, 71, 69, 57, 55, 54, and 41 can be attributed to the fragmentations indicated below:

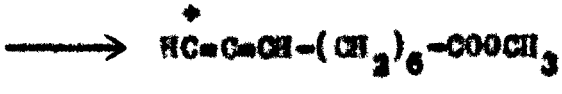
m/e 181

The ion peak at m/e 181 is very important for proving penultimate acetylenic structure. The appearance of this ion from the rearranged allenic acid can be rationalised according to Scheme 16.

Scheme 16







m/e 181

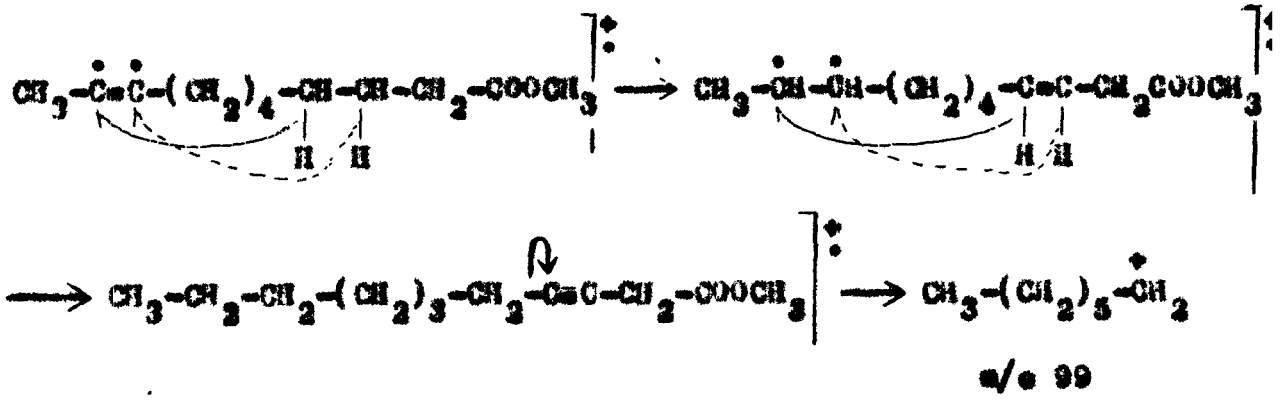
m/e 149 and 107

The ion peaks at m/e 149 and 107 are weak. These ions are formed by the loss of 32 amu (CH_3OH) and 74 amu (McLafferty rearrangement) from the ion m/e 181, respectively.

m/e 99

This fragment ion can be shown to arise from rearranged β,γ-acetylenic acid as shown in Scheme 17.

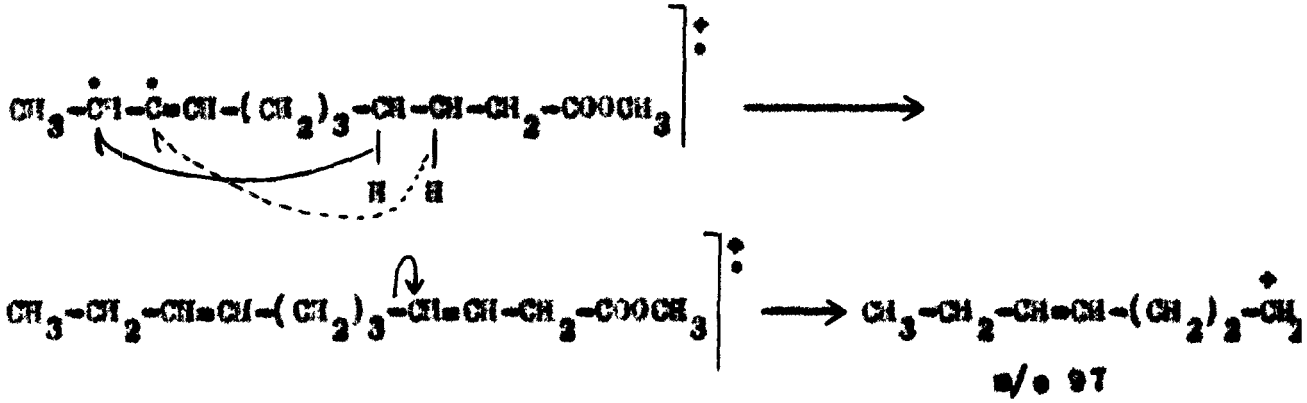
Scheme 17



m/e 97

The fragment ion m/e 97 probably originates from the rearranged allenic acid according to the mechanism shown in Scheme 18.

Scheme 18



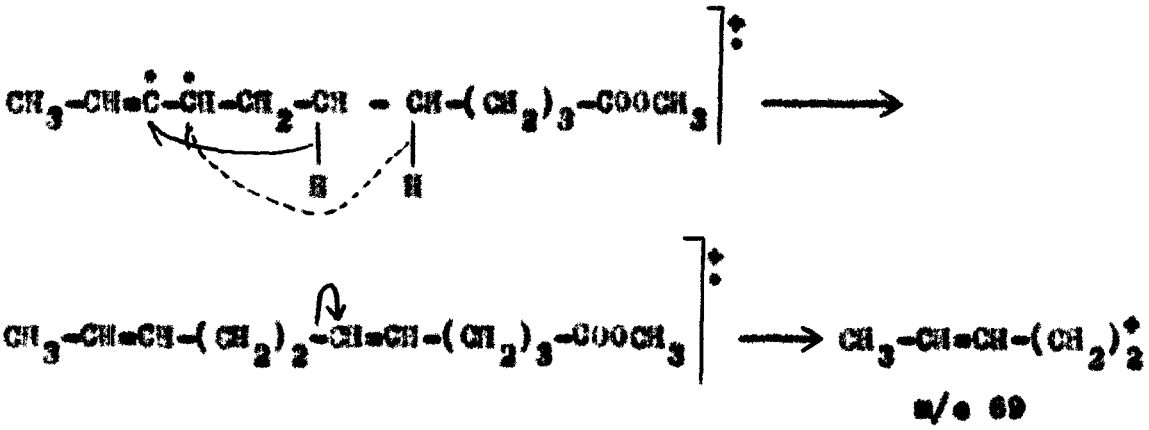
m/e 85 and 71

The ion peaks at m/e 85 and 71 obviously result by two consecutive CH_2 losses from the ion m/e 99, the latter being fairly strong.

m/e 69

The ion m/e 69 can possibly be arised from the rearranged allenic acid by the mechanism shown in Scheme 19.

Scheme 19

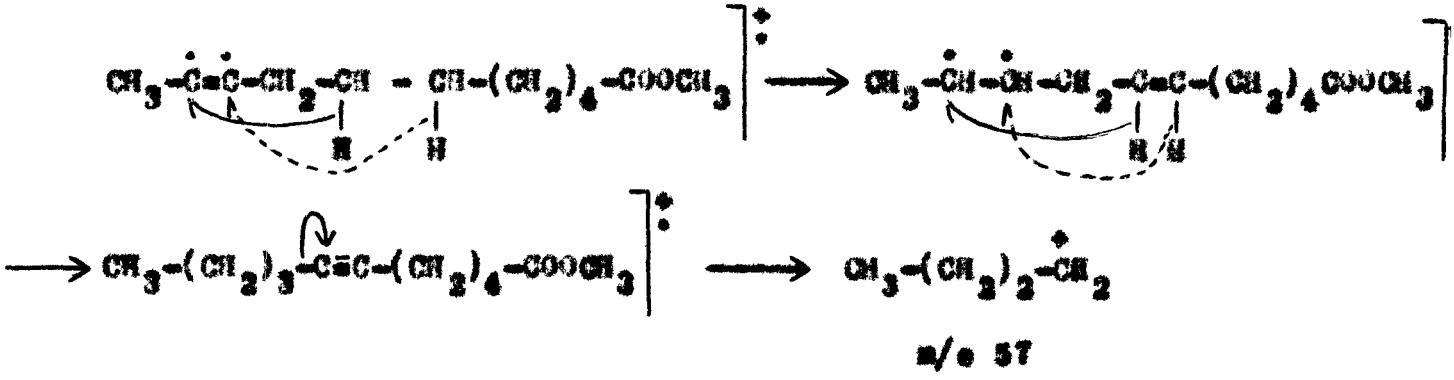


m/e 69

m/e 57

This fragment ion peak constitutes the base peak of the spectrum. The ion could be obtained by the loss of CH_2 from the fragment ion m/e 71. The formation of this ion can also be explained according to the mechanism depicted in Scheme 20.

Scheme 20



m/e 57

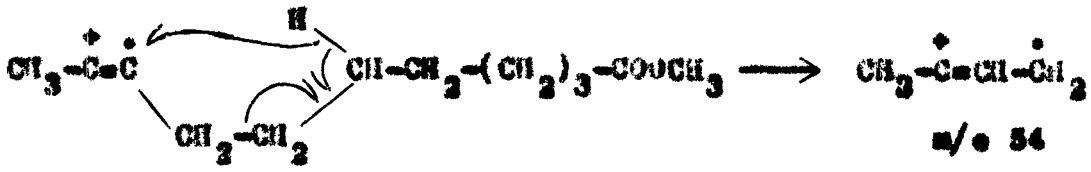
m/e 55

The fragment ion peak at m/e 55 is quite prominent and the corresponding ion results by the loss of CH_3 from the ion m/e 69.

m/e 54

The fragment ion m/e 54 is formed by McLafferty rearrangement (with respect to triple bond) as shown in Scheme 21.

Scheme 21



m/e 41

The ion peak at m/e 41 of medium intensity could possibly be obtained by the loss of CH_2 from the fragment ion m/e 55.

2.1. 9,10-Undecadienoic acid (XIII): A co-product during synthesis of 9-undecynoic acid (XIV)

A co-product occurring during the synthesis of 9-undecynoic acid (Scheme 13) by dehydrobromination of 10,11-dibromoundecanoic acid by aqueous potassium hydroxide, hitherto unreported, was isolated and characterised as 9,10-undecadienoic acid. Its

structure was established by combustion as well as by spectral methods. It is believed that the penultimate acetylenic acid (XIV) is formed from 10,11-dibromoundecanoic acid (XI) through the allenic intermediate (XIII).

Chromatographic fractionation of the crude product obtained by dehydrobromination of dibromoundecanoic acid (XI) by heating with aqueous potassium hydroxide afforded first fraction as allenic acid (XIII)(yield 15%). The acid (XIII) was a TLC homogenous liquid. Its elemental analysis corresponded to the molecular formula $C_{11}H_{18}O_2$. The IR spectrum of the methyl ester (XIIIA) (Figure 7) revealed characteristic bands at 1950s, 1710w and 850s (C=C=C str), and 1740s cm^{-1} (C=O str). The strong absorption at 1950 cm^{-1} in the spectrum was obtained as a result of the out-of-phase CCC stretch. The spectrum also gave a strong band at 850 cm^{-1} with its overtone at 1710 cm^{-1} which is arised by the terminal $=CH_2$ wagging vibration.[96] The NMR gave characteristic signals at δ 1.32 (10H, broad s, $-\underline{CH}_2-$), 2.05-2.50 (4H, overlapping signals, $>C=C=C-\underline{CH}_2-$ and $-\underline{CH}_2-COO$), 3.62 (3H, s, $-COO\underline{CH}_3$), 5.35 and 5.55 (2H, both m, $\underline{H}\diagdown C=C=C-$), and 6.06 (1H, m, $>C=C=\underline{CH}-$). The allenic methylene protons ($\underline{H}\diagdown C=C=C-$) are not similar stereochemically, therefore two different signals were found for them in NMR spectrum. The cis proton resonates at δ 5.35 and the trans proton at δ 5.55 both as multiplets. The multiplicity of both the signals as multiplet

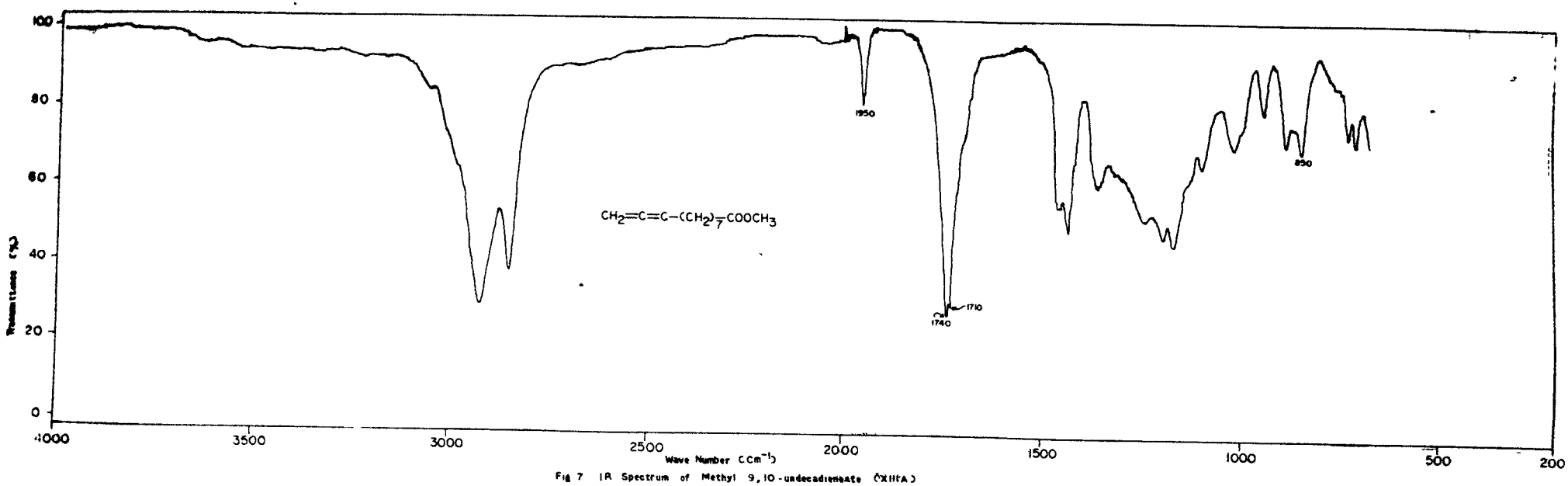


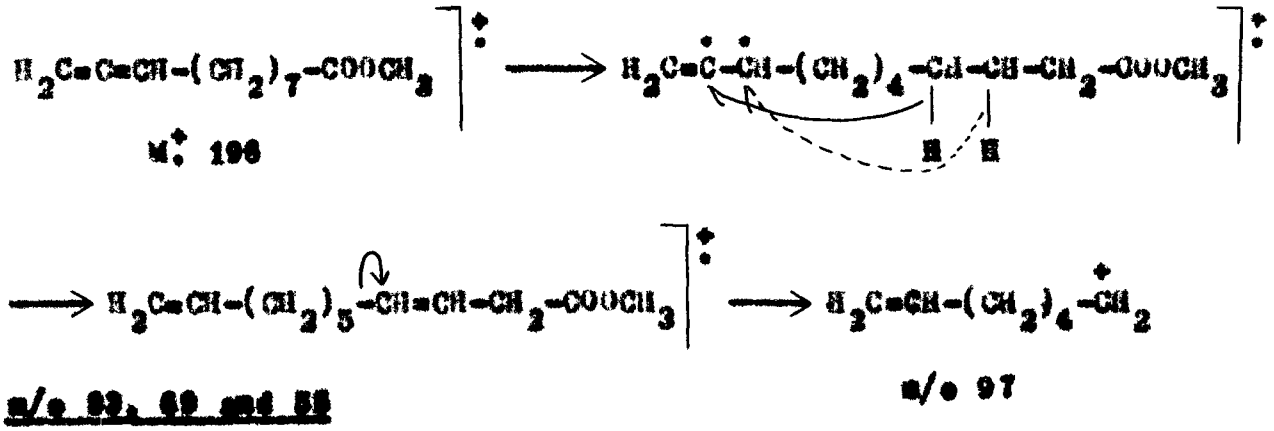
Fig 7 IR Spectrum of Methyl 9,10-undecadienoate (XIIIA)

may be due to coupling of terminal allenic methylene protons with each other and long range coupling with C-9 methine proton. Additional support in favour of the structure was obtained by mass spectrometry. The mass spectrum of XIIIA (Figure 8) showed peaks at m/e 197 (M+1) and 196 ($M^{+\cdot}$, $C_{12}H_{20}O_2$) along with associated peaks at m/e 165, 164, 137, 123, 122, 109, 95, 81 (base peak), 67, 53, and 39 in common as obtained in the spectra of acetylenic esters (XIIA and XIVA). Other salient ion peaks were observed at m/e 97, 83, 69, 55, 43, and 41. The formation of these fragment ions has been shown to occur according to following Schemes.

m/e 97

The fragment ion m/e 97 arises from the molecular ion after the rearrangements as shown in Scheme 22.

Scheme 22



m/e 83, 69 and 55

The ions m/e 83, 69, and 55 obviously result by consecutive losses of CH_3 from the ion m/e 97. The fragment ion peak

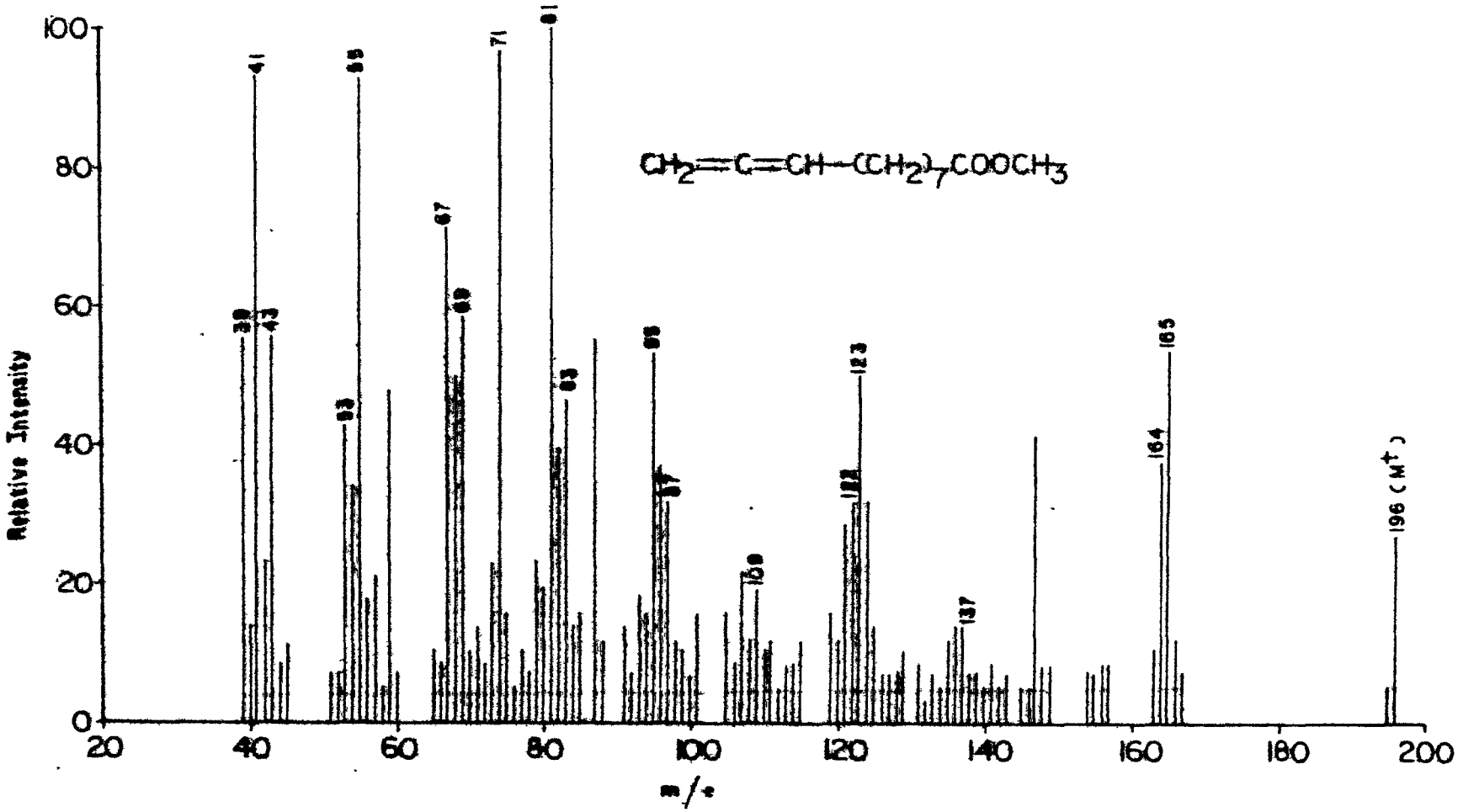


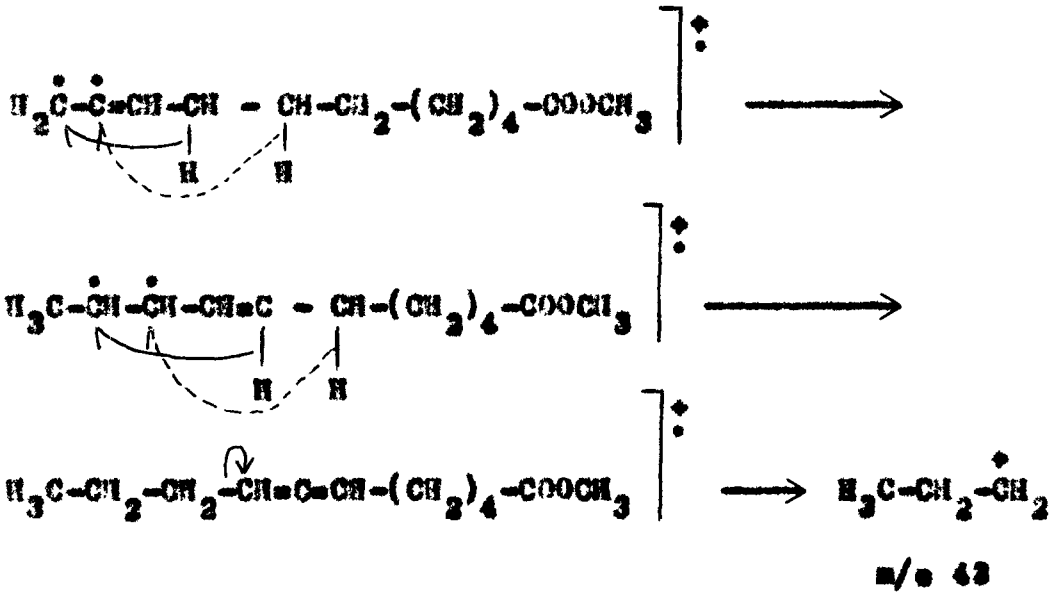
Fig 8. Mass Spectrum of Methyl 9, 10-undecadienoate (XIIIA)

at m/e 55 is fairly strong and this ion can also be obtained from the molecular ion according to the mechanism shown in Scheme 9.

m/e 43

This fragment ion could possibly be formed, probably following the way depicted in Scheme 23.

Scheme 23



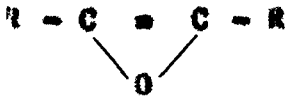
m/e 43

m/e 41

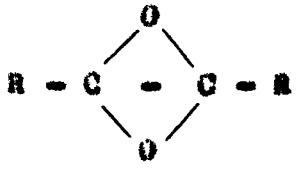
The ion peak at m/e 41 is quite prominent. This ion is obtained by the loss of CH_2 from the ion m/e 55.

3. Unusual and miscellaneous epoxidation studies of acetylenic fatty acids

The synthesis and chemistry of small-ring compounds has been and continues to be of interest to chemists. The simplest oxygen-containing heterocycle, oxirene (oxacyclopropene, XV) represents both an experimental and theoretical challenge as potential 4 π antiaromatic system. The history of oxirene is similar to that of many other unstable small-ring compounds. Its isolation was claimed and withdrawn several times during the past hundred years. Oxirenes have been shown in recent years to have transient existence and to be implicated as short-lived reactive intermediates in the peroxyacid oxidation of acetylenes[87-89] and in other acetylene oxidations,[90] in the addition of oxygen atom to acetylenes,[91] in the reaction of singlet methylene with carbon monoxide,[92] in the photolytic and thermal decomposition (Wolff rearrangement) of α-diazoketones[93] and ketenes,[94] and in the flash thermolysis of formal Diels-Alder adducts of oxirenes and arenes or dienes.[95] Each of these approaches involves formation of the heterocyclic ring from an acyclic precursor, a relatively unpromising route from a synthethic standpoint.



XV



XVI

R, H or alkyl or phenyl

Organic peroxyacid oxidations of phenyl- and diphenyl-acetylenes have been reported[87,88] which led to the formation of three different types of products. Each is explicable in terms of an oxirene intermediate reacting to give products (1) of further oxidation, (2) of rearrangement, and (3) of addition, depending on the reaction conditions. Further oxidation of oxirene intermediate (XV) has shown the formation of dioxabicyclo-butane (XVI). This dioxabicyclo derivative has also not yet been prepared or isolated by any route but shown to lead the isomerised products. It has been reported that acetylenes react considerably more slowly with peroxyacids than structurally similar olefins.[96]

Although the epoxidation of olefinic fatty acids with peroxyacids has been thoroughly studied, very little is known in the acetylenic acid series. Nikolajenak[24] and other workers[24,2] have tried the epoxidation of acetylenic linkage in methyl crepenynate with m-chloroperbenzoic acid but they did not succeed in obtaining the oxirene. With an objective of preparing oxirene and/or oxabicyclo derivatives, a new and interesting class of fatty acids, the perbenzoic acid (PBA) oxidation of methyl 9- and 10-undecynoates was investigated.

3.1. PBA oxidation of methyl 10-undecynoate (XIIA)

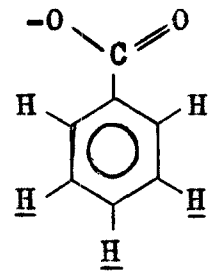
Reaction of methyl 10-undecynoate (XIIA) with perbenzoic acid was carried out in chloroform solution. No change in XIIA as evident by TLC, was observed when the reaction was conducted in

ice-bath. The reaction mixture was then kept at room temperature and progress of the reaction was monitored by TLC at intervals of 6 hr. After 24 hr direct TLC revealed two spots, R_f 0.91 and 0.84, the former being relatively strong and corresponded to unreacted methyl ester (XIIA). The faint spot at R_f 0.84 responded to positive picric acid TLC test[97] indicating the formation of an oxirene. In order to complete the epoxidation, the reaction mixture was left for 2 days. The product showed three more faint spots (R_f 0.68, 0.54, and 0.34) in addition to two spots already present (R_f 0.91 and 0.84) in TLC. Although at this stage the intensity of the spot (R_f 0.91) corresponding to starting material was lesser as compared to previous stage and the spot (R_f 0.84) still responded to positive picric acid TLC test but no enhancement in its intensity was observed. The spot corresponding to oxirene gradually disappeared and the intensity of the spots, developed on TLC plate due to other products formed by the reaction, increased with the increase in reaction time. During the initial stage of oxirene formation, all attempts to isolate the reaction product by column chromatography or by preparative TLC yielded unreacted acetylenic ester as a major product and two other products in negligible amounts which did not respond to picric acid TLC test. This showed that the oxirene is labile enough to undergo rearrangement in the column. Apparently, the negligible amounts of the rearranged products were due to very low yield of oxirene formed at this stage. Instead of the oxirene, final

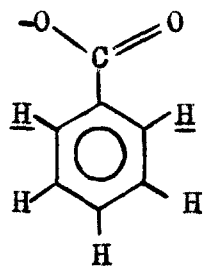
work-up of the reaction mixture after 5 days afforded a oily substance. This oily product showed four spots (R_f 0.91, 0.68, 0.54, and 0.34) on TLC plate with negative picric acid TLC test. Chromatographic separation of the crude product gave unreacted ester (XIIA) to the extent of 45% along with three other products XXII (16%), XXIII (10%), and XXIV (21%). First fraction obtained from the column was identified as unreacted XIIA by its TLC behaviour, elemental analysis, and IR and NMR spectra which were consistent with those of methyl 10-undecynoate.

Characterisation of XXII as methyl 11-aldehydo-10-benzoyloxyundecenoate

The elemental analysis of the product XXII corresponded to the formula $C_{19}H_{26}O_5$. Its IR spectrum gave bands at 1740-1720s,b (ester C=O and aldehyde C=O str), 1690s (aryl C=O str), and 1610m cm^{-1} (aromatic C=C str). The NMR spectrum gave signals at δ 1.22 (14H, broad s, $-C\underline{H}_2-$), 2.20 (2H, t, $-C\underline{H}_2-C(=O)-$), 3.55 (3H, s, $-COOC\underline{H}_3$), 4.16 (1H, m, $-C(=O)-C\underline{H}-$), 7.20 (3H, m,



), 7.90 (2H, d of d,



), and

9.25 (1H, d, $\underline{H}C(=O)-$).

Characterisation of XXIII as half methyl ester of 10-ethoxyundecene-11,1-dioic acid

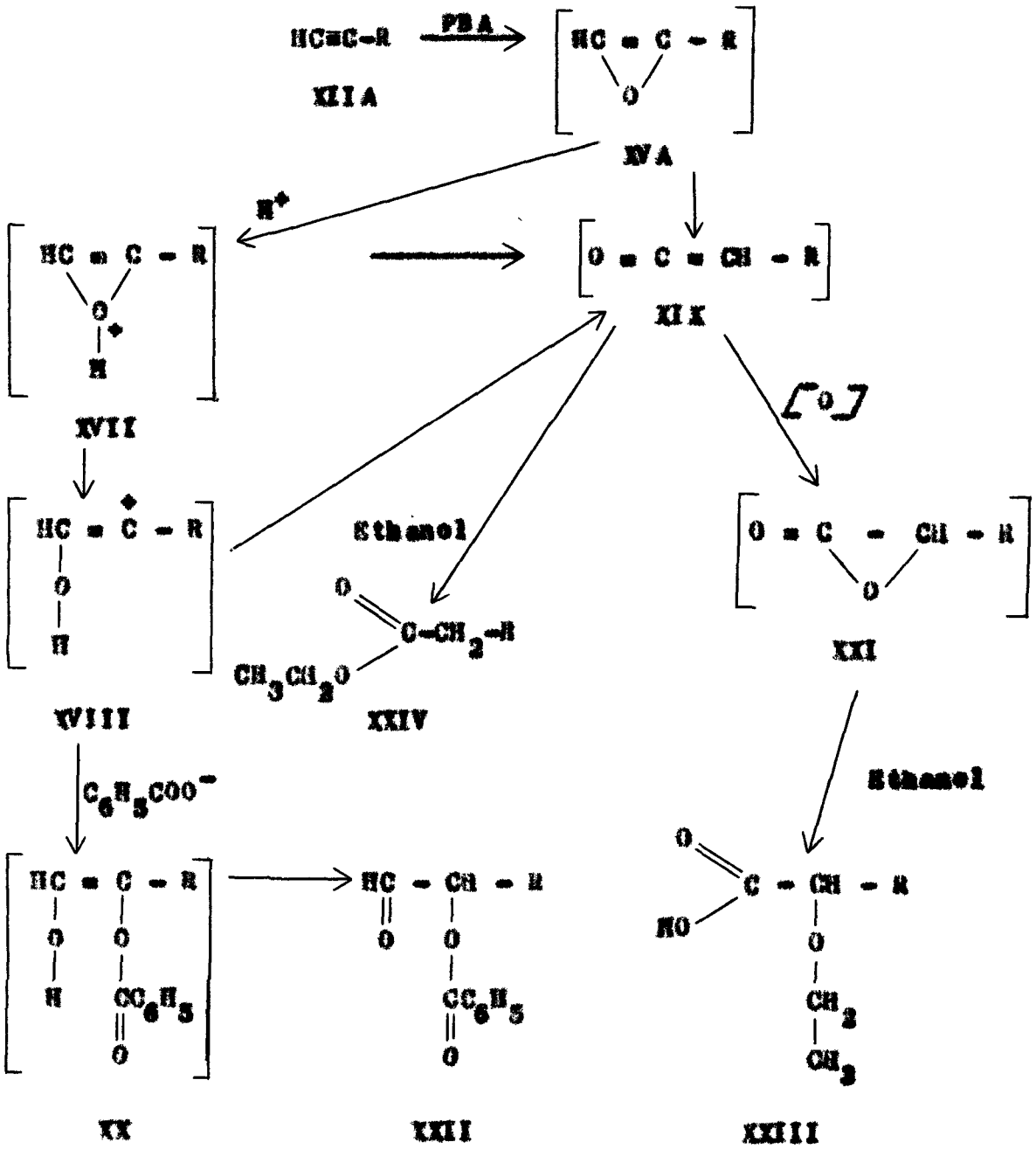
The product XXIII analysed for $C_{14}H_{26}O_5$. Its IR spectrum showed characteristic bands at 1740s (ester C=O str), 1710s (acid C=O str), and 1160m and 1080m cm^{-1} (C-O-C). The NMR spectrum gave the absorptions at δ 0.80 (3H, distorted t, $-OCH_2\underline{CH}_3$), 1.20 (14H, broad s, $-\underline{CH}_2-$), 2.20 (2H, t, $-\underline{CH}_2-COO$), 3.55 (3H, s, $-COO\underline{CH}_3$), 3.79 (1H, t, $OOC-\underset{O-}{\underline{CH}}-$), 4.05 (2H, t, $-O\underline{CH}_2CH_3$), and 10.10 (1H, s, $\underline{H}OOC-$, vanished in presence of D_2O).

Characterisation of XXIV as ethyl, methyl undecene-11,1-dioate

The microanalysis of the product XXIV revealed the molecular formula $C_{14}H_{26}O_4$. A broad and strong band in the carbonyl region (1750-1735 cm^{-1}) appeared in the IR spectrum which can be attributed to two ester carbonyl groups. The NMR spectrum showed signals at δ 0.84 (3H, distorted t, $\underline{CH}_3CH_2OOC-$), 1.25 (14H, broad s, $-\underline{CH}_2-$), 2.25 (4H, t like, $C_2H_5OOC-\underline{CH}_2-$ and $-\underline{CH}_2-COOCH_3$), 3.63 (3H, s, $-COO\underline{CH}_3$), and 4.24 (2H, t, $CH_3\underline{CH}_2OOC-$).

Scheme 24 depicts the formation of XXII, XXIII, and XXIV from the oxidation of methyl 10-undecynoate (XIIA) with PBA. The first step for the formation of all the three products is the addition of oxygen to the acetylenic bond to yield oxirene (XVA). Protonation of oxirene would produce cation (XVII).

Scheme 34



R, $-(CH_2)_8COOCH_3$

Oxirene has been predicted to be a strong base.[87] Immediately after the transfer of oxygen by perbenzoic acid to the acetylene, benzoic acid is available to the newly formed oxirene base, and inside the solvent cage. Addition of benzoate ion to protonated oxirene produced XX isolated as the α-aldehydo ester (XXII). Such type of addition product has also been reported by Stille and Whitehurst[87] in peroxyacid oxidation of diphenylacetylene. It has been shown[87,88] that, in the oxidation of acetylenes, rearrangement is one of the dominant features. Ketene formation was also reported in each instance via different intermediates. The formation of the rearranged products (XXIII and XXIV) in our case can also be attributed to the ketene intermediate (XIX). Two routes are plausible here for ketene (XIX) production. One route is migration of H from C-11 to C-10 in oxirene (XVA) by a concerted mechanism that could yield XIX directly. The alternate route to the ketene intermediate could arise from H migration in carbonium ion (XVIII) or from a concerted rearrangement in the protonated oxirene (XVII), both followed by proton elimination. The ketene, once produced, reacted with ethanol to form diester (XXIV). The ketene (XIX) also suffers further oxidation to produce an α-lactone (XXI) which on treatment with ethanol yielded ethoxy derivative of half ester of undecanedioic acid (XXIII). The ethanol is present since commercial chloroform (in which epoxidation carried out) contains about 1% of this alcohol as stabiliser and no attempt was made to remove it.

3.2. PBA oxidation of methyl 9-undecynoate (XIVA)

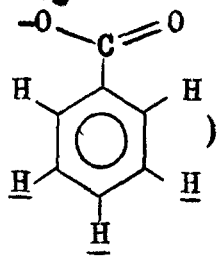
Similar treatment of methyl 9-undecynoate (XIVA) with perbenzoic acid in chloroform at room temperature for 9 days gave a mixture composed of 5% yield of XXXII, 13% yield of XXXIII, 9% yield of XXXIV, and 31% yield of XXXV. Reaction did not take place when it was carried out in ice-bath. After 24 hr direct TLC showed a faint spot (R_f 0.85) in addition to the spot (R_f 0.87) corresponding to the starting material. This faint spot of reaction product responded to picric acid TLC test[97] indicating the formation of an oxirene. The intensity of orange spot gradually decreased with the increase in reaction time. The oxirene defied all attempts of isolation. After usual work-up of the reaction mixture, the resulting oily product gave five spots (R_f 0.87, 0.83, 0.79, 0.52, and 0.39) in TLC with a major spot corresponding to the starting material. Column fractionation of crude product yielded unreacted methyl 9-undecynoate (XIVA, 39%), XXXII (5%), XXXIII (13%), XXXIV (9%), and XXXV (31%). No fraction was responded to picric acid TLC test. First fraction obtained from the column was characterised as unreacted acetylenic ester by comparison of its TLC behaviour, elemental analysis, and IR and NMR spectra with those of authentic XIVA. The product XXXIII could not be identified.

Characterisation of XXXII as half methyl ester of 9-methoxy-9-methyldecane-10,1-dioic acid

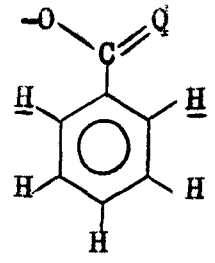
The product XXXII analysed for $C_{13}H_{24}O_5$. Its IR spectrum showed bands at 1740-1720s,b (ester C=O and acid C=O str), and 1170m and 1090m cm^{-1} (C-O-C). The NMR spectrum gave characteristic signals at δ 1.27 (12H, broad, s, $-CH_2-$), 1.71 (3H, s, $HOOC-\overset{O}{\overset{|}{C}}-\overset{CH_3}{\overset{|}{}}$), 2.28 (2H, t, $-CH_2-COO$), 3.68 (3H, s, $-COOCH_3$), 3.78 (3H, s, $HOOC-\overset{OCH_3}{\overset{|}{C}}-$), and 10.10 (1H, s, HOOC-, disappeared upon addition of D_2O).

Characterisation of XXXIV as methyl 8-ethylcarboxy-(1'-benzoyloxy)-octenoate

The microanalysis of the product XXXIV revealed the molecular formula $C_{19}H_{26}O_6$. Its IR spectrum gave bands at 1740-1700s,b (ethylcarboxy C=O, ester C=O, and aryl C=O str), and 1600m and 1580m cm^{-1} (aromatic C=C str). The NMR spectrum showed signals at δ 1.24 (10H, broad s, $-CH_2-$), 1.48 (3H, d, $-O-\overset{CH_3}{\overset{|}{C}}-O-$), 2.30 (4H, m, $-O-\overset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2-COO$), 3.67 (3H, s, $-COOCH_3$), 5.32 (1H, q, $-O-\overset{H}{\overset{|}{C}}-O-$), 7.51 (3H, m,



), and 8.60 (2H, d of d,



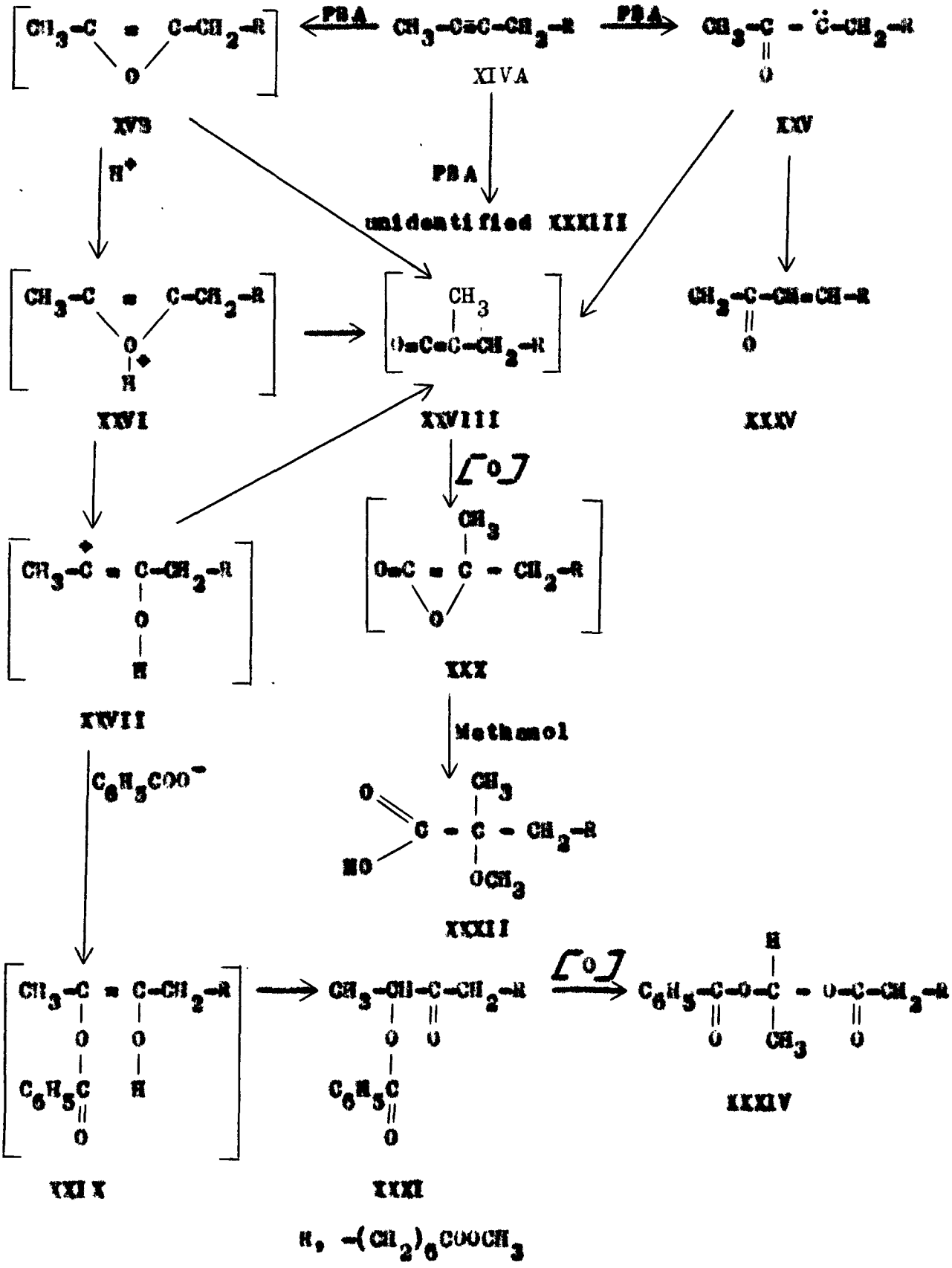
).

Characterisation of XXXV as methyl 10-oxo-cis-8-undecenoate

The elemental analysis of the product XXXV corresponded to the molecular formula $C_{12}H_{20}O_3$. It responded to positive DNP test.[98] Its IR spectrum revealed characteristic bands at 1740s (ester C=O str), 1720s (oxo C=O str), and 1620m cm^{-1} (cis C=C str). The NMR spectrum gave signals at δ 1.29 (10H, broad s, $-CH_2-$), 2.21 (3H, s, $CH_3-C(=O)-$), 2.27 (2H, t, $-CH_2-COO$), 3.65 (3H, s, $-COOCH_3$), 6.0 (1H, d, $-C(=O)-CH=CH-$), and 6.76 (1H, m, $-C(=O)-CH=CH-$).

The reaction mechanism (Scheme 25) similar to that described in peroxidation of terminal acetylenic ester (Scheme 24) seems to be reasonable to explain the products of peroxidation of the penultimate acetylenic ester (XIVA). Examination of Scheme 25 shows that the products XXXII and XXXIV are economically explicable by an oxirene intermediate (XVB). α-Lactone (XXX), obtained by further oxidation of ketene (XXVIII), may react with methanol to produce methoxy derivative of branched-chain decenedioic acid (XXXII). The methanol is present in the reaction mixture from the preparation of the solution of perbenzoic acid.[99a] The formation of such type of product is evident from the literature concerning peroxidation of phenylacetylene with PBA.[100] McDonald and Schwab[100] have reported that the formation of methyl phenylacetate arises from the

Schema 25


PBA
PBA
XIVA
XVB
XXV
H+
PBA
unidentified XXXIII
XXVI
XXVIII
XXXV
[O]
XXX
XXVII
Methanol
C6H5COO-
XXXII
[O]
XXIX
XXXI
XXXIV
R, -(CH2)6COOCH3

reaction of the intermediate phenylketene with methanol present in PBA solution. The formation of product XXXIV may be explained by a Baeyer-Villiger type oxidation of α-keto ester (XXXI) which is produced after the addition of benzoate ion to the oxirene (XVB) as shown in the Scheme. α,β-Unsaturated ketone (XXXV) resulted from the acetylenic ester (XIVA) through the intermediate XXV.

Although the oxirene (XVA or XVB) was not isolated, the products of perbenzoic acid oxidation of methyl 9- and 10-undecynoates can be most conveniently accounted for as arising from this intermediate. Apparently, the rate-controlling step for the formation of all products is the addition of the first mole of oxygen to the acetylenic bond. This is indicated by the fact that in both instances large amounts of starting material were recovered.

4. Hypobromination of acetylenic fatty acids

The hypohalogenation studies on internal olefinic fatty acids were made previously.[101-104] The products formed were usually an isomeric inseparable mixture of internal halohydrins. The inseparability of the mixture of the isomeric halohydrins suggested that the location of the halohydroxy function in the middle of the fatty acid chain may result in the formation of an eutectic mixture not separable by fractional crystallisation or chromatography. Champetier et al.[105] reported the addition of hypobromous acid to 10-undecenoic acid to yield exclusively 11-bromo-10-hydroxyundecanoic acid. Recently, a reinvestigation[33b] in our laboratory on the addition of hypobromous acid to 10-undecenoic acid led to the formation of isomeric mixture of 11(10)-bromo-10(11)-hydroxyundecanoic acids. Although the isomeric mixture of the terminal halohydrins could not be separated by column chromatography, but analytical TLC clearly showed the presence of two bromohydrins having closer R_f values and these were confirmed after chemical modification. This was contrary to the earlier observation that one bromohydrin is exclusively formed during the hypobromination of 10-undecenoic acid. Ansari et al.[106] from author's laboratory carried out a systematic study of the hypohalogenation of long-chain α,β-unsaturated acids. The resulting mixture of 2(3)-halo-3(2)-hydroxy acids has been successfully resolved by column

chromatography. The amenability of 2(3)-halo-3(2)-hydroxy acids to chromatographic separation was ascribed to the effect of ester group on the polarity of the halohydroxy function. Very recently, the hypohalogenation of β-hydroxyolefinic (ricinoleic) acid also carried out in author's laboratory has shown the formation of halohydroxy and cyclic ether derivatives.[107]

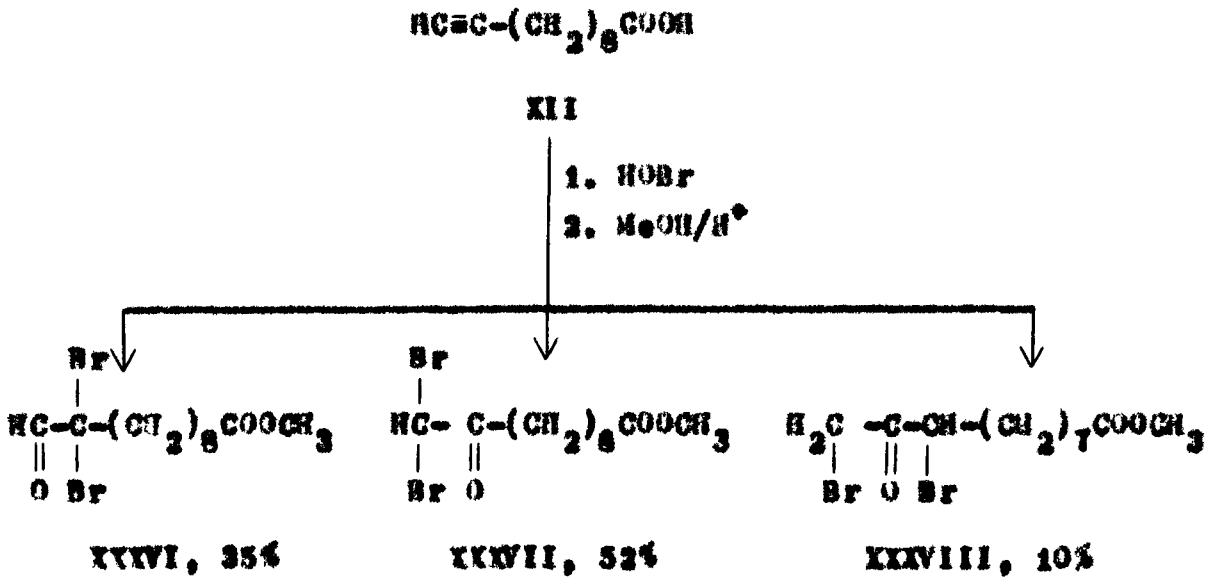
Hypohalogenation of acetylenic fatty acids has not been reported and this led us to carry out the reaction with 9- and 10-undecynoic acids. The hypobromination of these terminal and penultimate acetylenic acids afforded separable isomeric α-dibromoketone derivatives. The mode of addition of hypobromous acid ($\overset{-}{HO}\overset{+}{Br}$) to acetylenic acids and the nature of reaction products are of considerable theoretical interest. Therefore hypobromination was chosen for present study.

4.1. Addition of HOBr to 10-undecynoic acid (XII)

The reaction carried out in the present investigation (Table I) involves the addition of freshly prepared solution of sodium hypobromite to 10-undecynoic acid (XII). The resulting products were isolated following the procedure of King.[102] The mixture after methylation yielded a brown syrupy liquid which showed three distinct spots on analytical TLC plate. Column chromatography of the crude material over silica gel gave three TLC homogenous products, two major (XXXVI and XXXVII) and one

minor (XXXVIII). The reaction products were characterised by elemental analysis and spectral data.

Table I



Characterisation of product XXXVI as methyl 11-aldehyde-10,10-dibromoundecanoate

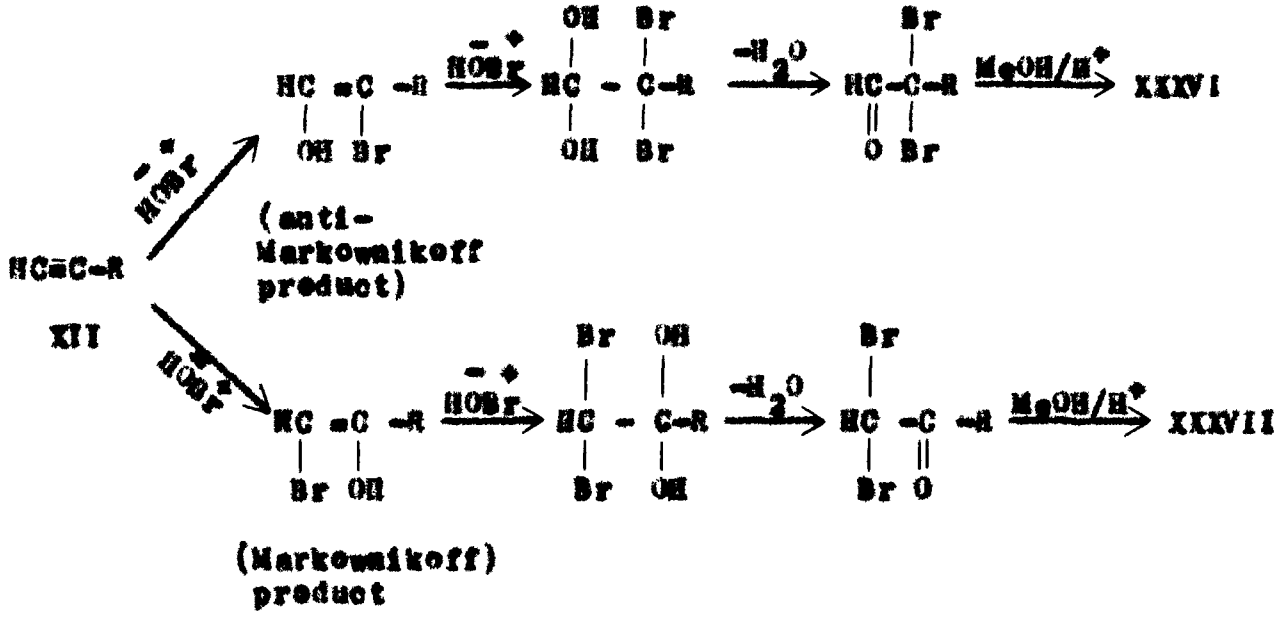
The compound XXXVI analysed for $C_{12}H_{20}O_3Br_2$. It responded to Beilstein test.[99b] Its IR spectrum gave bands at 1740s (ester C=O str), 1730s (aldehyde C=O str), and 730s cm^{-1} (C-Br str). The NMR spectrum gave signals at δ 1.36 (12H, broad s, $-\underline{CH}_2-$), 2.23 (2H, t like, $-\underline{CH}_2-COO$), 2.68 (2H, t, $-\underset{\underset{Br}{|}}{\overset{\overset{Br}{|}}{C}}-\underline{CH}_2-$), 3.61 (3H, s, $-COO\underline{CH}_3$), and 9.30 (1H, s, $\underline{HC}(=O)-$).

Characterisation of product XXXVII as methyl 11,11-dibromo-10-oxoundecanoate

The elemental analysis of the compound XXXVII corresponded to the formula $C_{12}H_{20}O_3Br_2$. It gave positive Beilstein and DNP[98] tests. Its IR spectrum gave bands at 1735s (ester C=O str), 1720s (exo C=O str), and 720s cm^{-1} (C-Br str). The NMR spectrum showed signals at δ 1.38 (12H, broad s, $-CH_2-$), 2.24 (4H, t, $-\underset{\underset{O}{\|}}{C}-\underset{\underset{Br}{|}}{CH_2}-$ and $-CH_2-COO$), 3.62 (3H, s, $-COOCH_3$), and 6.09 (1H, s, $\underset{\underset{Br}{|}}{HC}-$).

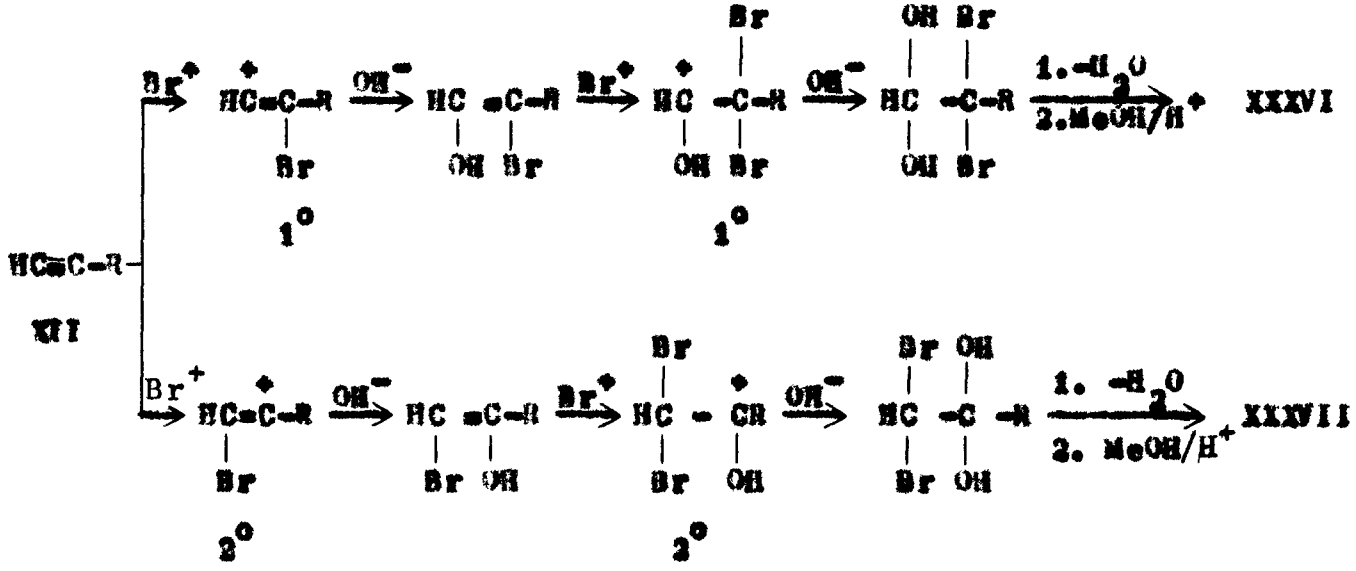
The formation of α gem-dibromoaldehyde (XXXVI) and α gem-dibromoketone (XXXVII) derivatives can be rationalised in terms of the mechanistic sequence shown in Scheme 26. Addition of two molecules of HOBr to acetylenic acid takes place in two steps to form the isomeric products.

Scheme 26



Two mechanisms can be considered for the addition of HOBr to acetylenic linkage. The first molecular addition, involves synchronous attachment of HOBr across the acetylenic bond with cis stereospecificity. The second Ad_E2 (addition, electrophilic, bimolecular)[108] which involves stepwise addition of OH^- and Br^+ to the triple bond via a carbonium ion intermediate. We obtained methyl 11,11-dibromo-10-oxoundecanoate (XXXVII) in comparatively greater amount than methyl 11-aldehydo-10,10-dibromoundecanoate (XXXVI). This could be explained in terms of more stability of 2^0 carbonium ions formed through Ad_E2 mechanism (Scheme 27).

Scheme 27



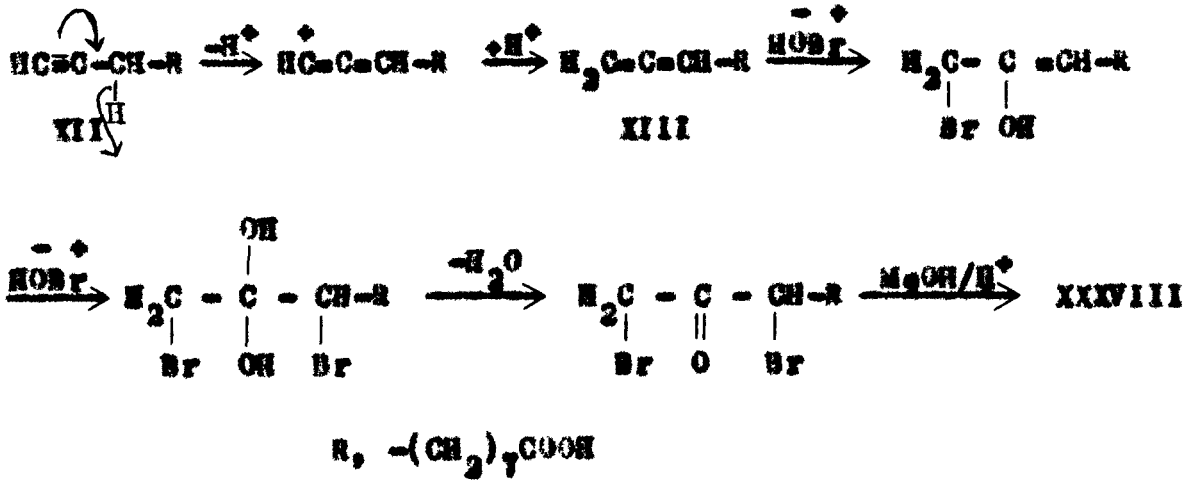
R, $-(CH_2)_8COOH$

Characterisation of product XXXVIII as methyl 9,11-dibromo-10-oxoundecanoate

The microanalysis of the compound XXXVIII revealed the molecular formula $C_{12}H_{20}O_3Br_2$. It responded to Beilstein and DNP tests. Its IR spectrum showed characteristic bands at 1750s (ester C=O str), 1735s (oxo C=O str), and 720s cm^{-1} (C-Br str). The NMR spectrum gave the absorptions at δ 1.22 (12H, broad s, $-\underline{CH}_2-$), 2.20 (2H, t, $-\underline{CH}_2-COO$), 3.66 (3H, s, $-COO\underline{CH}_3$), 3.81 (2H, s, $\underline{H}_2C(Br)-$), and 4.22 (1H, m, $-\underline{CH}(Br)-$).

This non-vicinal dihaloketone derivative (XXXVIII) may be formed by the addition of two molecules of HOBr to the rearranged allenic acid (XIII) as shown in Scheme 28.

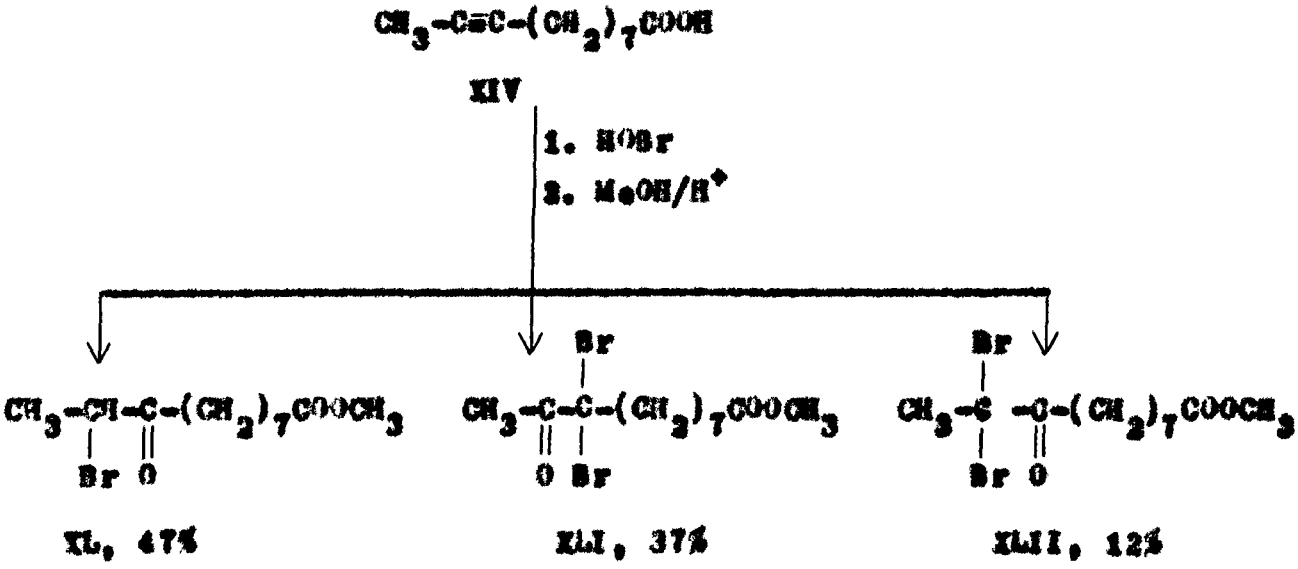
Scheme 28



4.2. Addition of HOBr to 9-undecynoic acid (XIV)

Similar addition of freshly prepared solution of sodium hypobromite to 9-undecynoic acid (XIV) was carried out. The product after methylation was obtained as brown syrupy liquid which showed three distinct spots in direct TLC. Column chromatographic fractionation of the crude material afforded three TLC homogeneous products XL, XLI, and XLII (Table II). The structures of the compounds were deduced by elemental analysis, and by IR and NMR spectroscopies.

Table II



XL, 47% XLI, 37% XLII, 12%

Characterisation of product XL as methyl 10-bromo-9-oxoundecanoate

The microanalysis of the compound XL revealed the molecular formula $C_{12}H_{21}O_3Br$. It gave positive Beilstein and

DNP tests. Its IR spectrum showed bands at 1755s (ester C=O str), 1720s (oxo C=O str), and 720s cm^{-1} (C-Br str). The NMR spectrum gave characteristic signals at δ 1.31 (10H, broad s, $-CH_2-$), 2.23 (4H, t, $-\overset{\overset{O}{\|}}{C}-CH_2-$ and $-CH_2-COO$), 2.61 (3H, d, $CH_3-\overset{\overset{Br}{|}}{C}-$), 3.10 (1H, q, $-\overset{\overset{Br}{|}}{CH}-$), and 3.61 (3H, s, $-COOCH_3$). The signal as doublet due to the terminal methyl protons appeared at δ 2.61 instead of the usual δ 0.90 because of the deshielding effect of Br attached to neighbouring carbon (C-10).

Characterisation of product XLI as methyl 9,9-dibromo-10-oxoundecanoate

The elemental analysis of XLI corresponded to the molecular formula $C_{12}H_{20}O_3Br_2$. Beilstein and DNP tests were positive. Its IR spectrum showed bands at 1740s (ester C=O str), 1710s (oxo C=O str), and 720s cm^{-1} (C-Br str). The NMR spectrum gave signals at δ 1.30 (10H, broad s, $-CH_2-$), 2.24 (2H, t like, $-CH_2-COO$), 2.45 (3H, s, $CH_3-\overset{\overset{O}{\|}}{C}-$), 2.67 (2H, t, $-\overset{\overset{Br}{|}}{C}-CH_2-$), and 3.63 (3H, s, $-COOCH_3$).

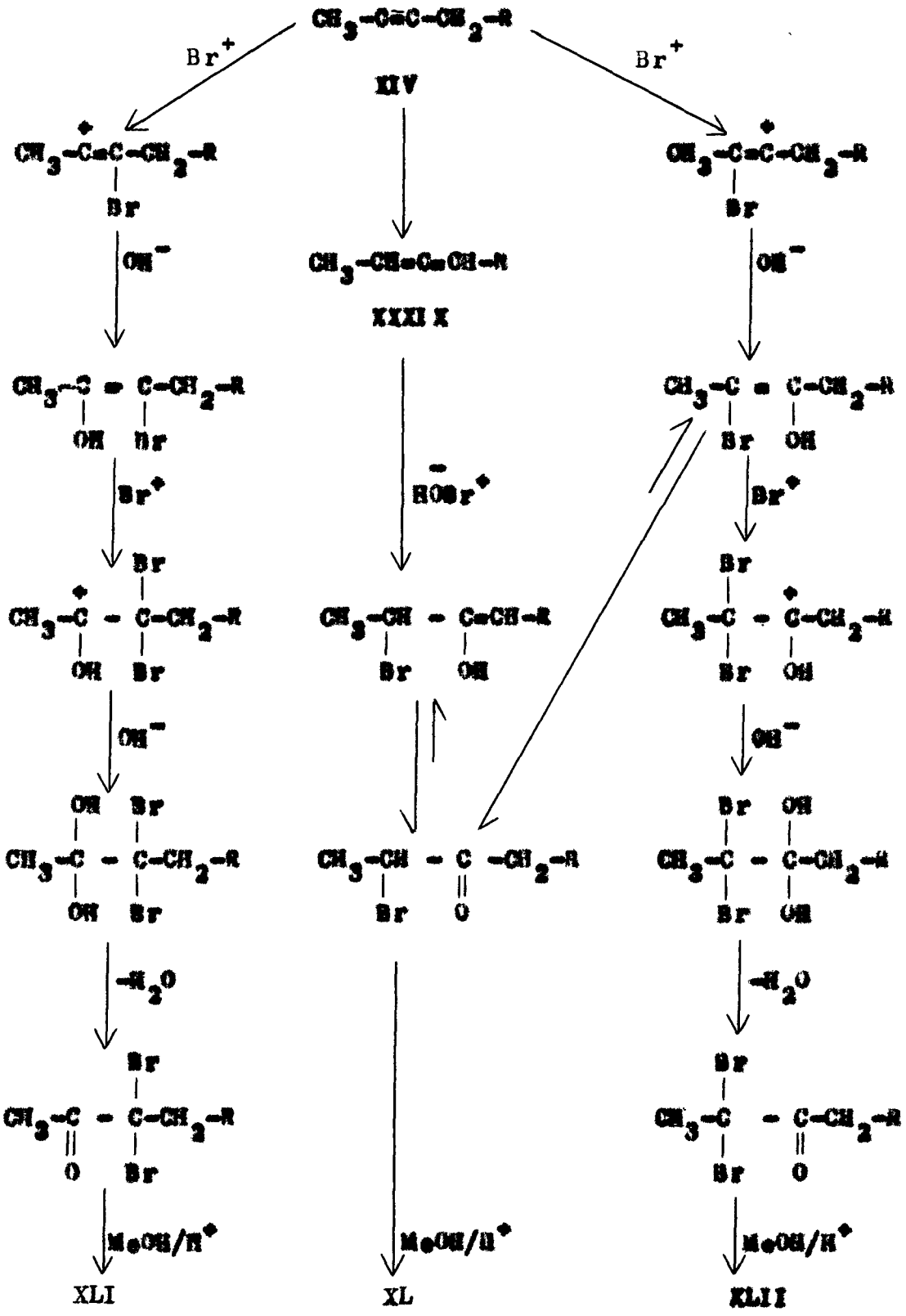
Characterisation of product XLII as methyl 10,10-dibromo-9-oxoundecanoate

The compound XLII analysed for $C_{12}H_{20}O_3Br_2$. It gave positive Beilstein and DNP tests. Its IR spectrum revealed

characteristic bands at 1740s (ester C=O str), 1725s (oxo C=O str), and 730s cm^{-1} (C-Br str). The NMR spectrum gave characteristic signals at δ 1.32(10H, broad s, $-CH_2-$), 3.24 (4H, t, $-\overset{\overset{O}{\|}}{C}-CH_2-$ and $-CH_2-COO$), 3.51 (3H, s, $CH_3-\overset{\overset{Br}{|}}{\underset{\underset{Br}{|}}{C}}-$), and 3.62 (3H, s, $-COOCH_3$).

The mechanism shown in Scheme 20 explains the formation of α-bromoketone (XL) and α gem-dibromoketone (XLI and XLII) derivatives by hypobromination of penultimate undecynoic acid (XIV). Adopting Ad_E2 mechanism,[108] the stepwise addition of two molecules of HOBr to XIV yielded the pair of 9(10), 9(10)-dibromo-10(9)-oxoundecanoates (XLI and XLII). The relatively large amount of the product XLI to XLII obtained could be explained in terms of the greater stability of the carbonium ion of the former due to the attachment of electron releasing (CH_3) group to the charge-bearing carbon atom (C-10). Since the carbonium ion corresponding to XLII is less stable, it tends to rapid keto-enol tautomerisation to yield XL. The product XL may also be formed by the addition of one molecule of HOBr to the allenic acid (XXXIX), a rearranged product of XIV.

Scheme 20


CH3-C≡C-CH2-R
XIV
Br+
Br+
OH-
CH3-CH=C=CH-R
XXXIX
-HOBr+
Br+
OH-
Br+
OH-
-H2O
-H2O
MeOH/H+
MeOH/H+
MeOH/H+
XLI
XL
XLII
R, -(CH2)6COOH

5. Preparation and reactions of vinyl β-iodoazide derivatives of acetylenic fatty acids

Organic azides have been of recent interest in photochemistry,[109] in cycloadditions,[110] and as precursors to amine functions.[111] Furthermore the ambident and amphoteric character of the azide function is indicated by its participation in electrophilic as well as nucleophilic reactions. The synthetic chemist has at his disposal a variety of methods for the stereospecific introduction of oxygen functions into the carbon skeleton, e.g., via opening of epoxides, hydroboration of olefins, or reduction of ketones. Until recently the same has not been true for functional groups containing nitrogen. Generally the organic azides are prepared from unsaturated compounds by the addition of a pseudohalogen, iodine azide (IN_3) to the carbon-carbon multiple bond in a highly stereospecific trans manner.[112]

The addition of iodine azide to olefinic fatty acids conducted in our laboratory has been shown to be useful in determining stereoselectivity as well as regioselectivity in addition reactions. Thus terminal and α,β-unsaturated acids led exclusively to the regiospecific products, whereas internal cis/trans olefinic acids give the opposite regioisomers.[113] Addition of IN_3 to β- and γ-hydroxyolefinic fatty acids[113] has also been studied in author's laboratory. In former case an inseparable mixture of two regioisomers was formed with no

hydroxyl group participation, whereas, in later case the reaction proceeded in an intramolecular manner to yield a 1,4-epoxide as major product. Haasner et al.[114] investigated the addition of IN_3 to acetylenes and discovered that 1-phenylpropyne reacted with IN_3 in the opposite regiochemical sense than an acid-catalysed hydration leading to 2-azido-1-iodo-1-phenylpropene. No report is available about IN_3 addition to acetylenic fatty acids. Thus, with a view to study both stereochemistry and orientation in additions to acetylenic acids, it was considered worthwhile to investigate this reaction on terminal and penultimate undecynoic acids.

5.1. Addition of IN_3 to methyl 10-undecynoate (XIIA)

For the preparation of vinyl β-iodoazide derivative of methyl 10-undecynoate (XIIA) the procedure of Fowler et al.[115] was adopted which is illustrated in Scheme 30. The progress of the reaction was monitored by TLC. The spot due to vinyl iodoazide (XLV) appeared just below the spot due to XIIA.

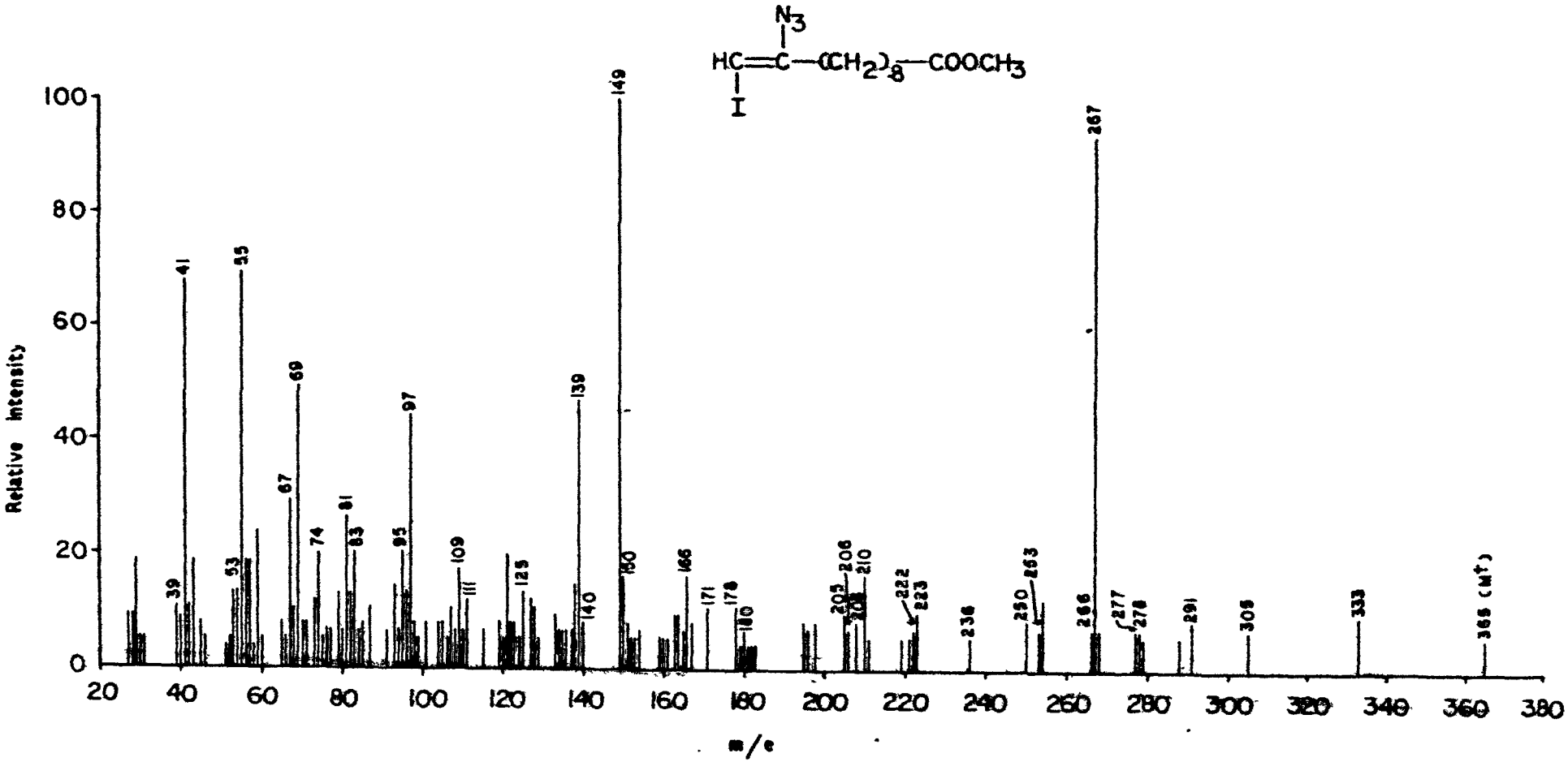


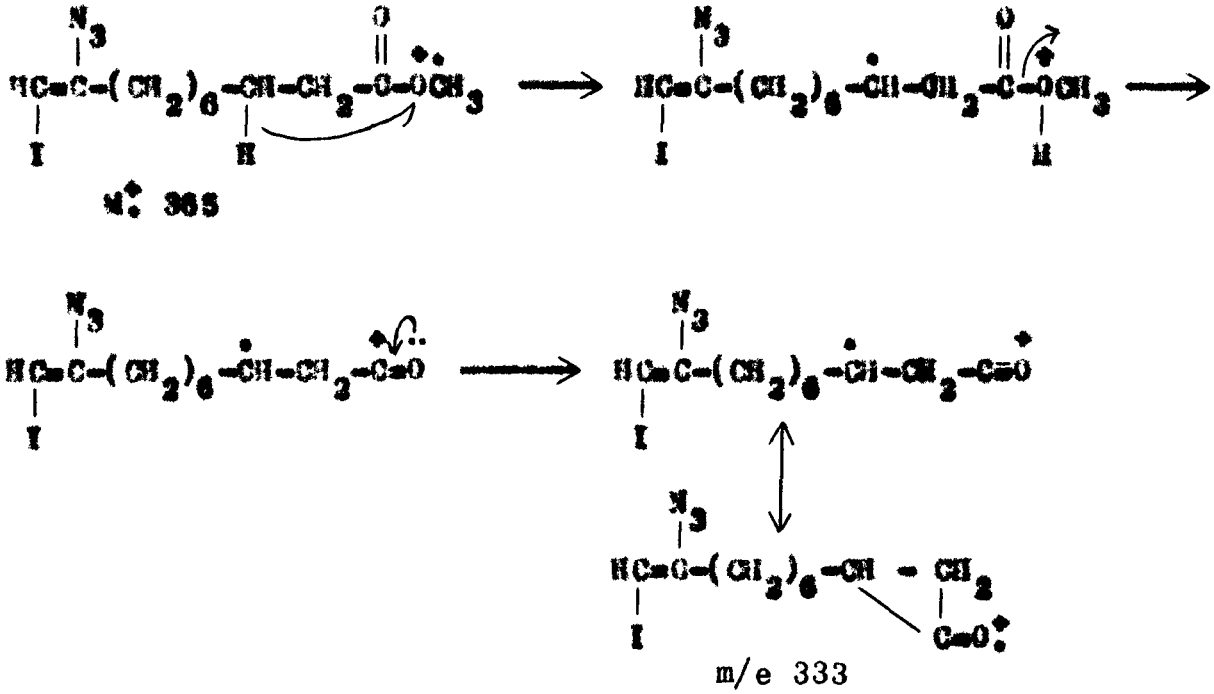
Fig 10- Mass Spectrum of Methyl 10-azide-11-iodoundecenoate (XLV)

253, 250, 236, 223, 222, 210, 208, 206, 205, 198, 195, 180, 178, 171, 166, 150, 140, 140, 139, 137, 125, 123, 122, 121, 109, 97, 95, 83, 81, 79, 74, 69, 68, 67, 55, 53, 43, 41, 40, and 39. The formation of important ions has been suggested to occur according to the Schemes given below:

m/e 333 ($M-CH_3OH$)

This fragment ion obviously results by the loss of methanol from the molecular ion (Scheme 31).

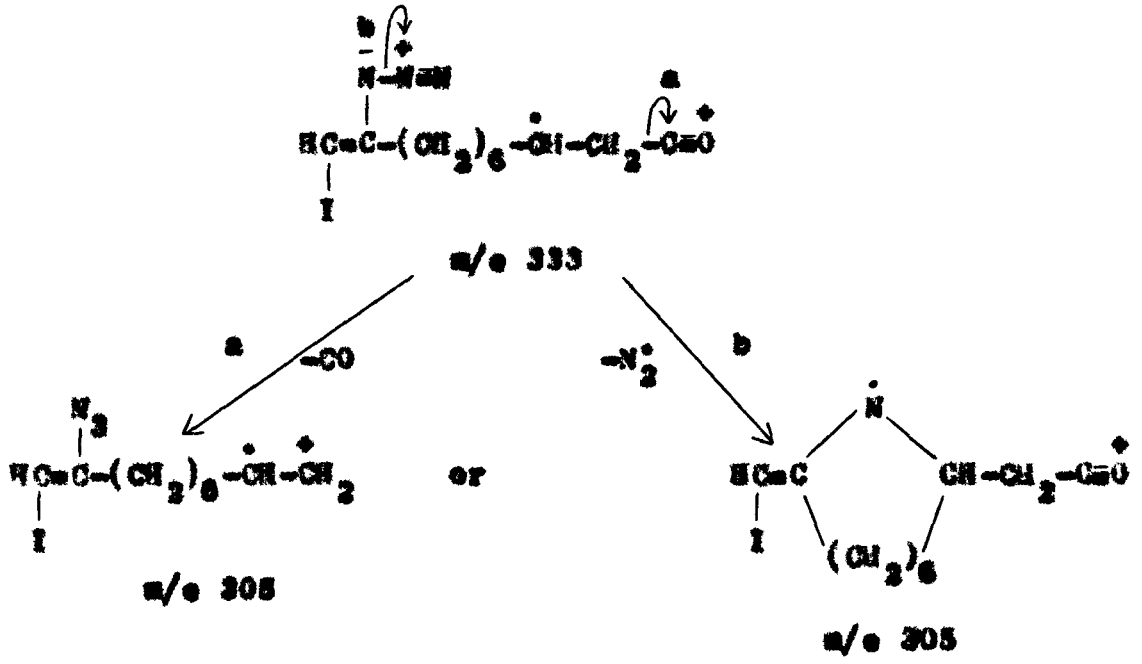
Scheme 31



m/e 305 (m/e 333-CO or N_2)

The ion m/e 305 originates from the ion m/e 333 by the loss of CO (a) or N_2 (b). Both the possibilities have been considered in Scheme 32.

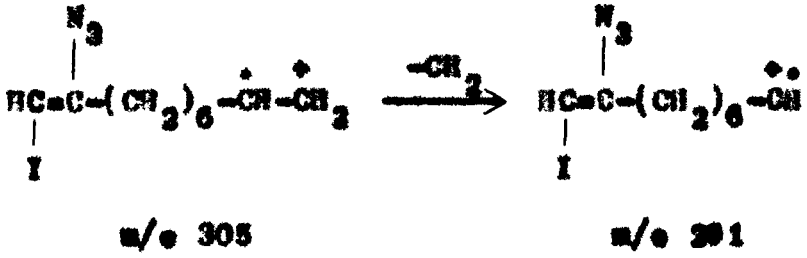
Scheme 32



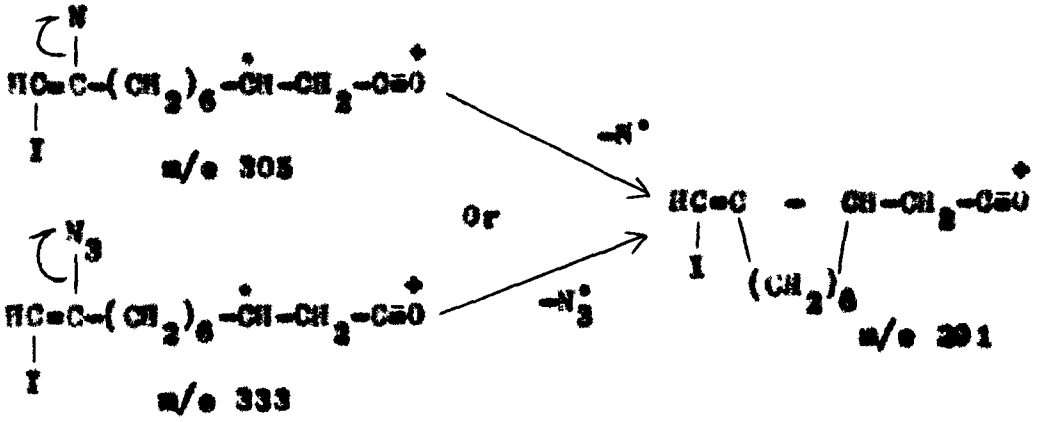
m/e 291

This ion apparently results by the loss of CH_2 or N from the ion m/e 305. The ion can also be obtained by the loss of N_3 from the ion m/e 333. The path ways are depicted in Scheme 33.

Scheme 33



Or



m/e 305

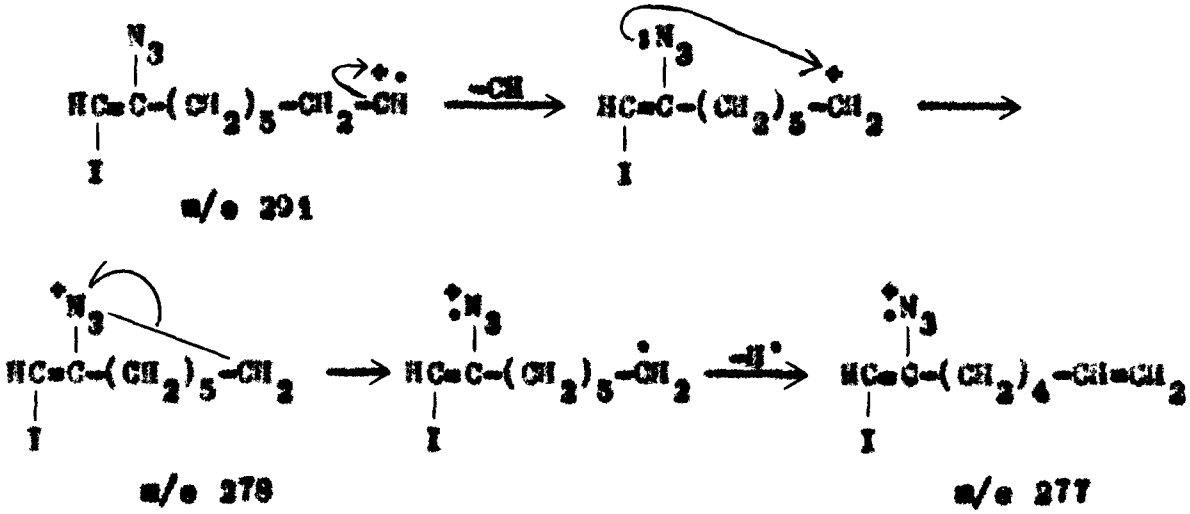
m/e 333

m/e 291

m/e 278 and 277 (m/e 291-CH) (278-H)

The loss of CH and then H from the fragment ion m/e 291 leading to the ions m/e 278 and 277 can be shown according to Scheme 34.

Scheme 34



m/e 291

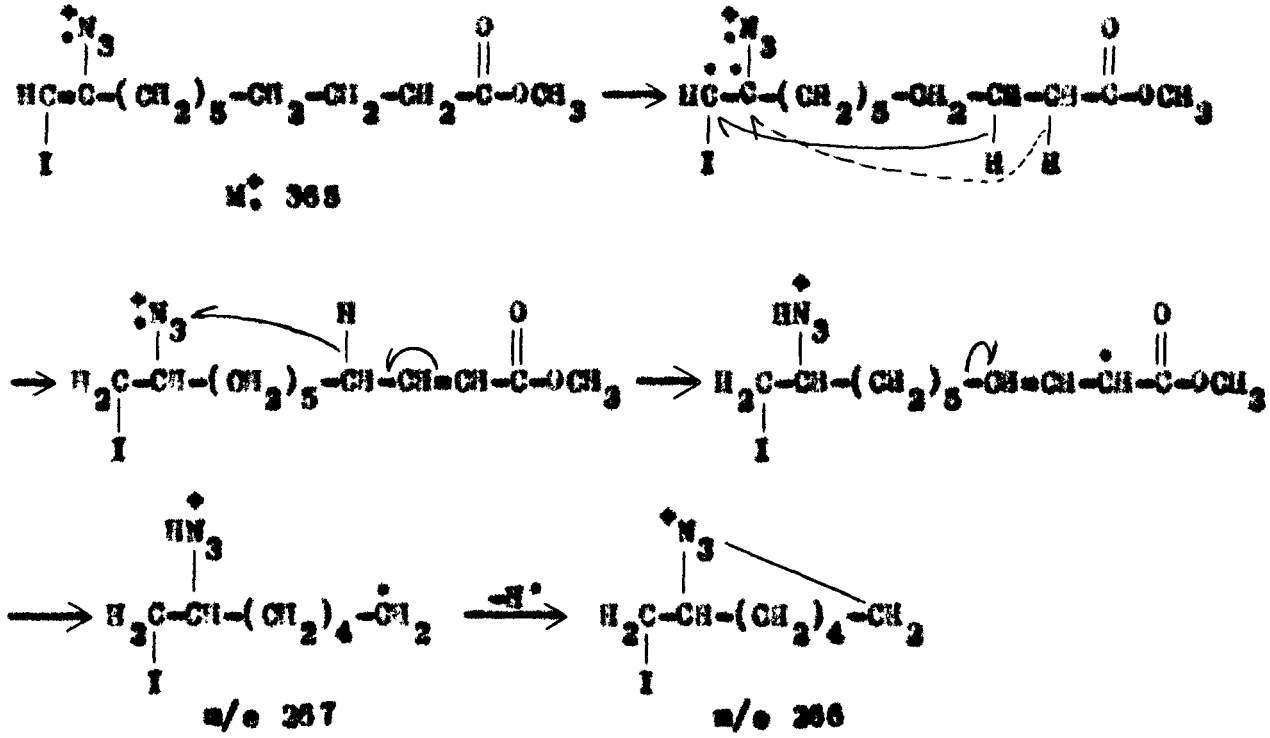
m/e 278

m/e 277

m/e 267 and 266

The ion peak at m/e 267 is very prominent. The ions m/e 267 and 266 could possibly be obtained from the molecular ion according to Scheme 35.

Scheme 35



m/e 267 → m/e 266

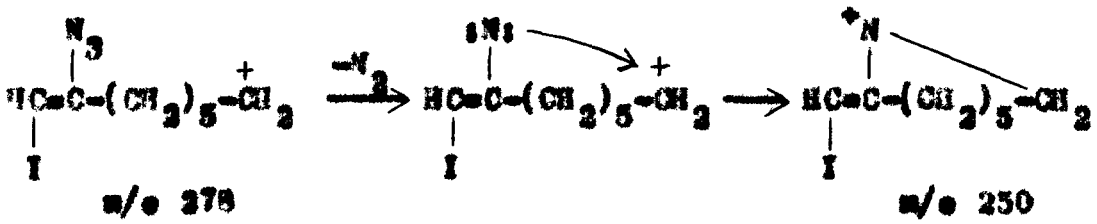
m/e 253

The fragment ion m/e 253 is derived from the ion m/e 267 by the loss of CH_2.

m/e 250

The formation of this ion can be rationalised by the loss of N_2 from the ion m/e 278 (Scheme 36).

Scheme 36

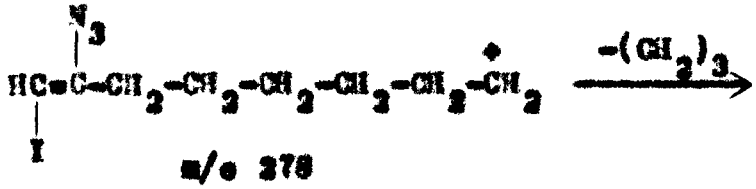


m/e 278 → m/e 250

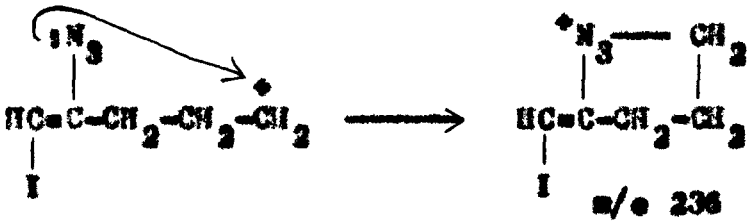
m/e 236

The fragment ion m/e 236 apparently results by the loss of C_3H_6 or N_3 from the fragment ion m/e 278. Both the possibilities have been discussed in Scheme 37.

Scheme 37

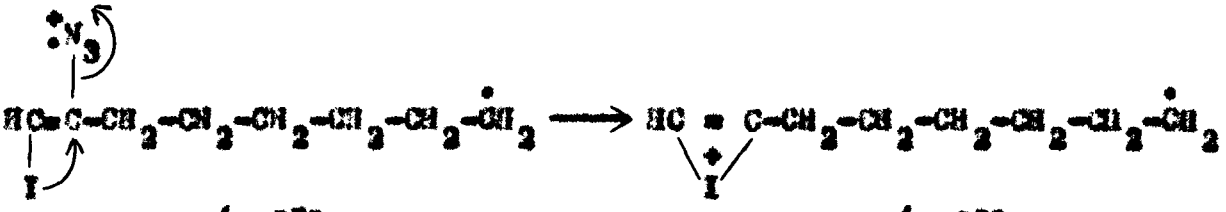


m/e 278



m/e 236

Or



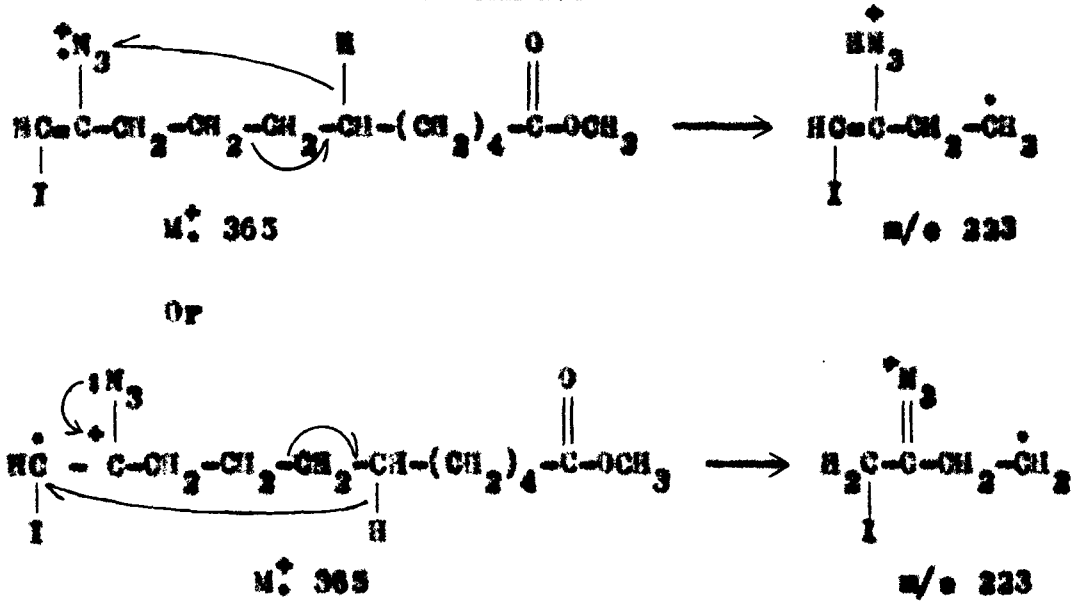
m/e 278 m/e 236

This ion can also be obtained by the loss of CH_2 from the ion m/e 250.

m/e 223

The following mechanisms have been proposed to account for the origin of ion m/e 223 (Scheme 38).

Scheme 38



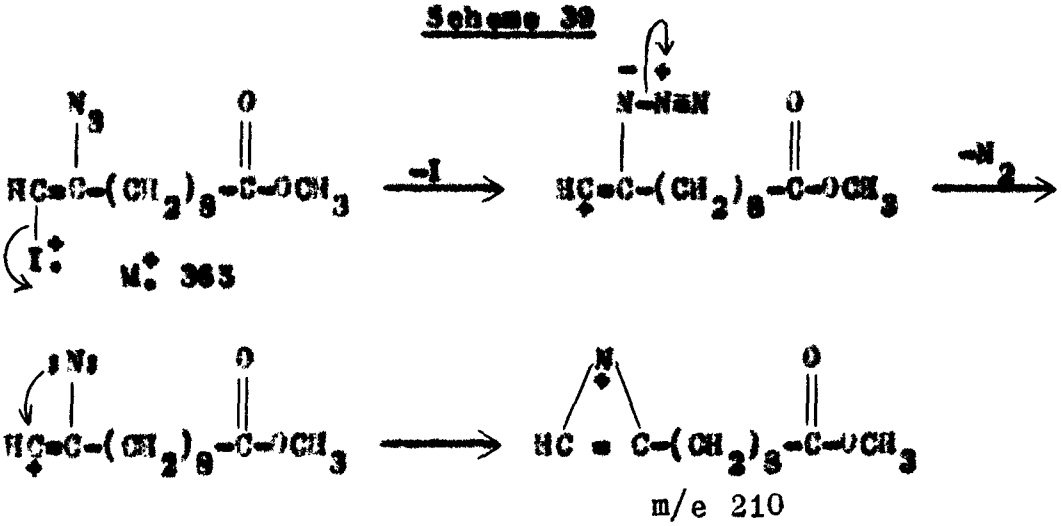
m/e 222

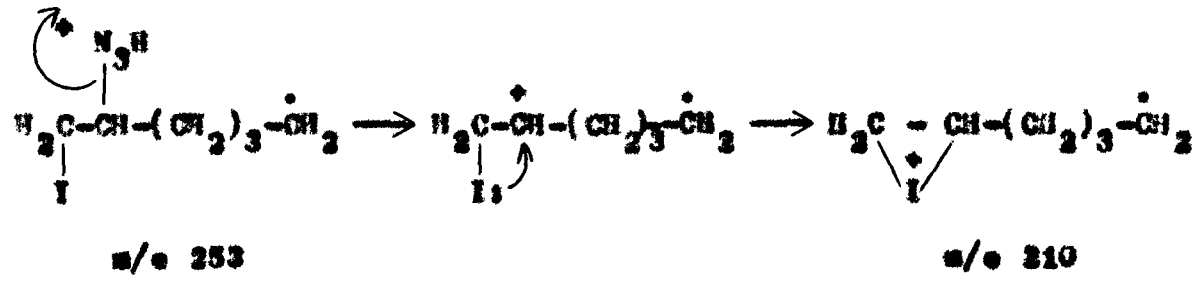
The ion m/e 222 is derived from the ion m/e 236 by the loss of CH_2.

m/e 210

The fragment results by the loss of I and N_2 from the molecular ion or by the loss of 43 amu from the ion m/e 253 as depicted in Scheme 39.

Scheme 39





m/e 253 m/e 210

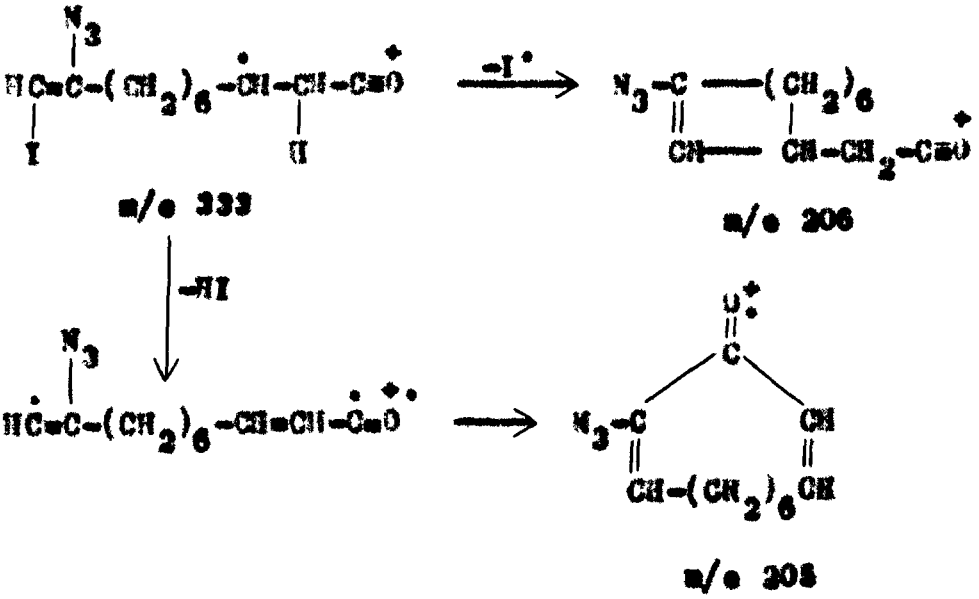
m/e 209

The ion m/e 209 obviously results by the loss of CH_2 from the ion m/e 222.

m/e 206 and 205

The ions m/e 206 and 205 most probably originate by the loss of I and HI, respectively, from the ion m/e 333 (Scheme 40) as it happens in case of many iodo compounds.

Scheme 40



m/e 333 m/e 206

m/e 205

m/e 180

The ion peak at m/e 180 was observed as doublet. The ion can be originated by the loss of N_2 or C_2H_4 from the ion m/e 208.

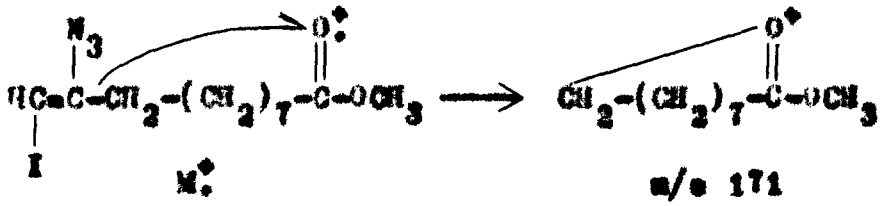
m/e 178

The ion m/e 178 originates by the loss of CH_3OH from the ion m/e 210.

m/e 171

This ion may arise from molecular ion by cleavage between C-9 and C-10 (Scheme 41).

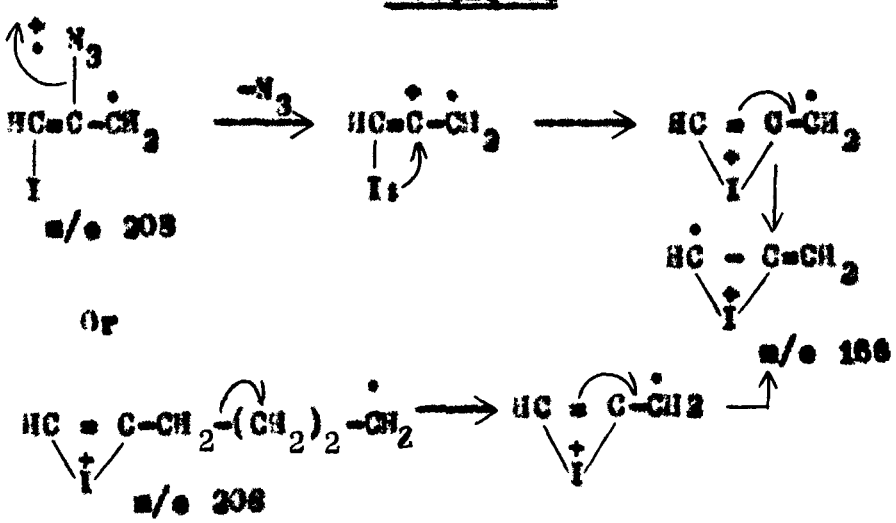
Scheme 41



m/e 166 (m/e 208-N_3 or C_3H_6)

The formation of the ion m/e 166 can be shown according to Scheme 42.

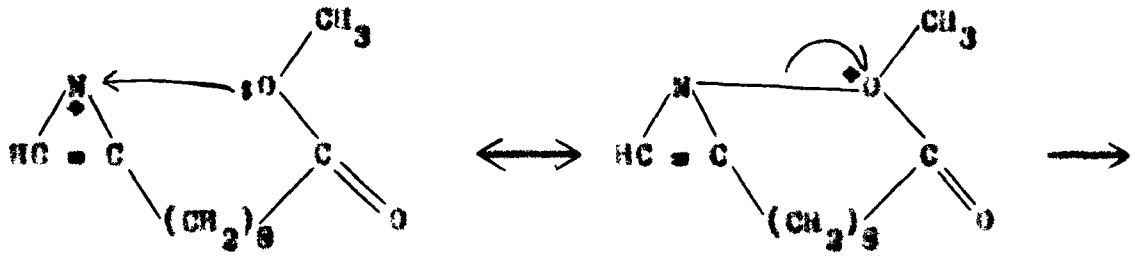
Scheme 42



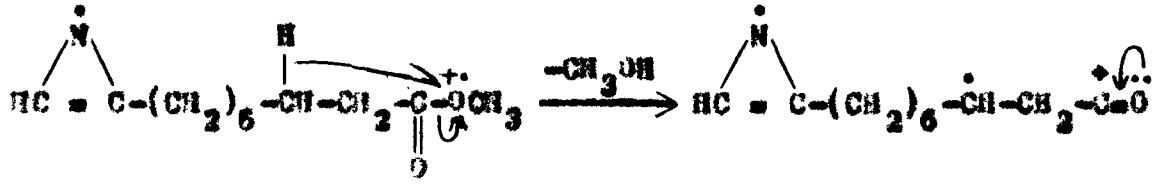
m/e 150 and 149

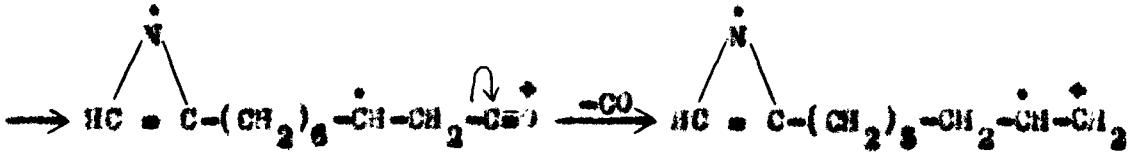
The ion peak m/e 149 constitutes the base peak of the spectrum. The fragment ions m/e 150 and 149 could possibly be obtained from the ion m/e 178 according to the mechanism shown in Scheme 43.

Scheme 43



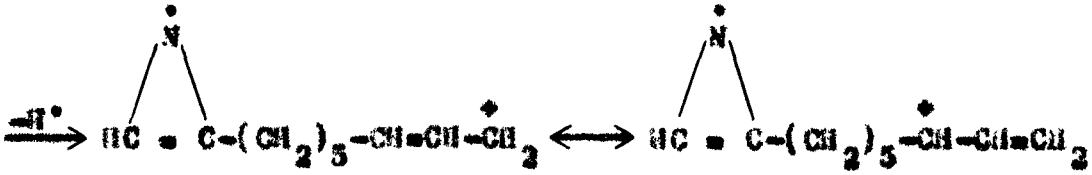
m/e 210





m/e 178

m/e 150

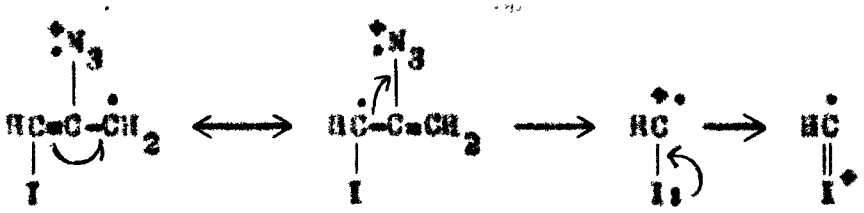


m/e 149

m/e 140

This fragment ion can conveniently be shown as arising from the ion m/e 208 (Scheme 44).

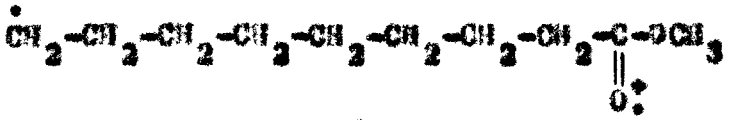
Scheme 44



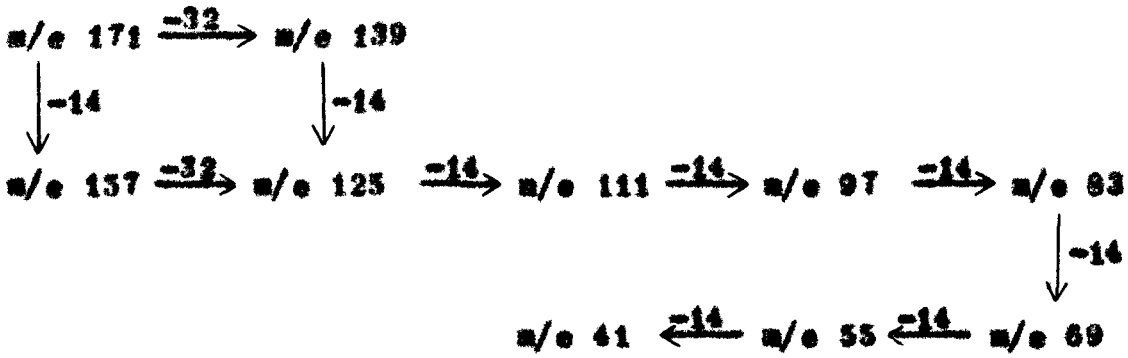
m/e 208 m/e 140

The formation of other characteristic ions m/e 139, 125, 111, 109, 97, 95, 83, 81, 69, 67, 55, 53, 41, and 39 has been proposed according to the sequences illustrated in Schemes 45 and 46.

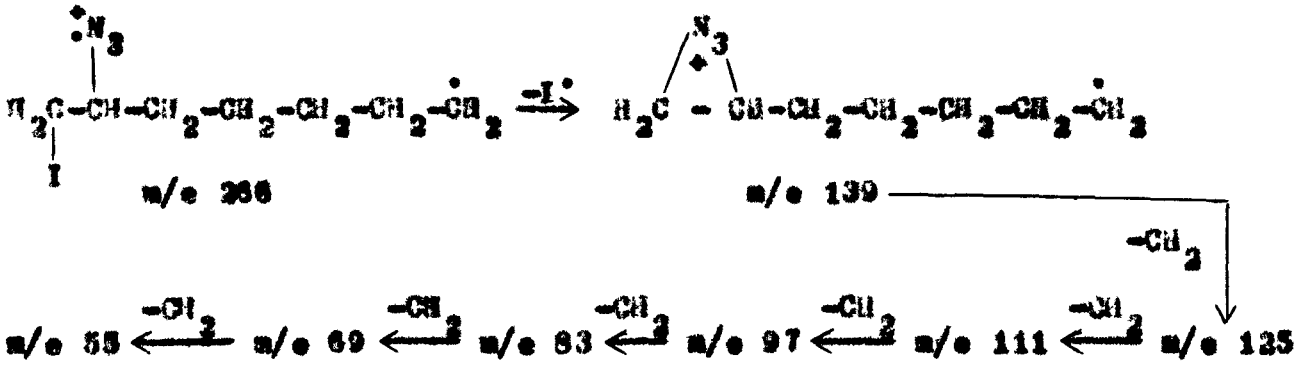
Scheme 45



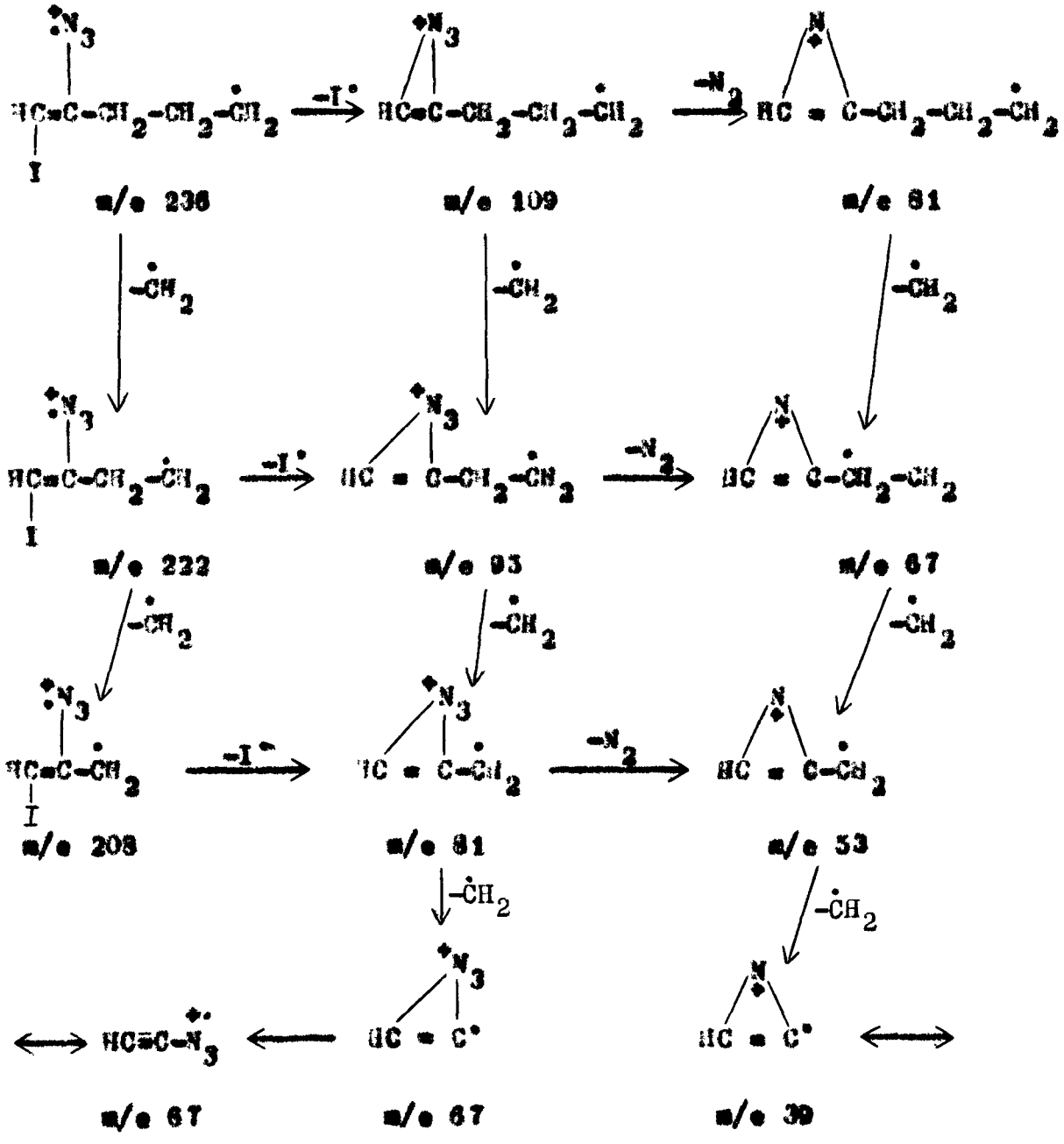
m/e 171



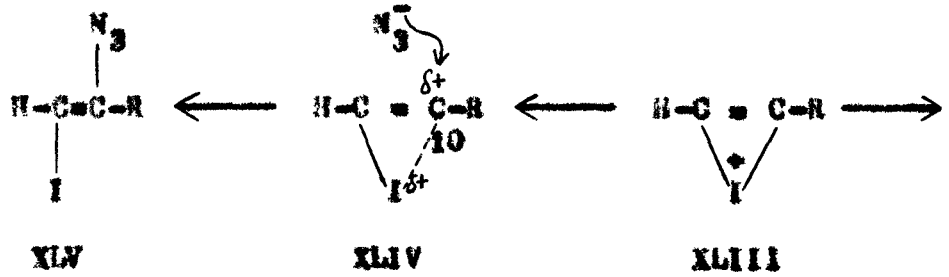
Or


m/e 266
m/e 139
-CH2
m/e 55
m/e 69
m/e 83
m/e 97
m/e 111
m/e 125

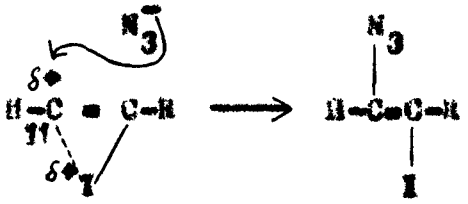
Scheme 46


m/e 236
m/e 109
m/e 81
m/e 222
m/e 93
m/e 67
m/e 208
m/e 81
m/e 53
m/e 67
m/e 67
m/e 39

The regiospecific adduct formation can be explained with the help of a mechanism involving the regiochemistry of a reaction. The best possible mechanism for the IN_3 addition to terminal acetylene is consistent with the intermediacy of a three-membered ring iodonium ion,[112,114] XLIII, opening of which proceeds via the transition state (XLIV or XLVI).



XLV XLIV XLIII



XLVI XLVII

R, $-(CH_2)_8COOCH_3$

It appears that somehow a substituent such as R in XLIII stabilises the positive charge on C-10 to such an extent that its dominance and subsequent attack by the nucleophile (N_3^-) leads exclusively to XLV via the transition state XLIV, though on steric grounds one would have favoured the formation of XLVII.

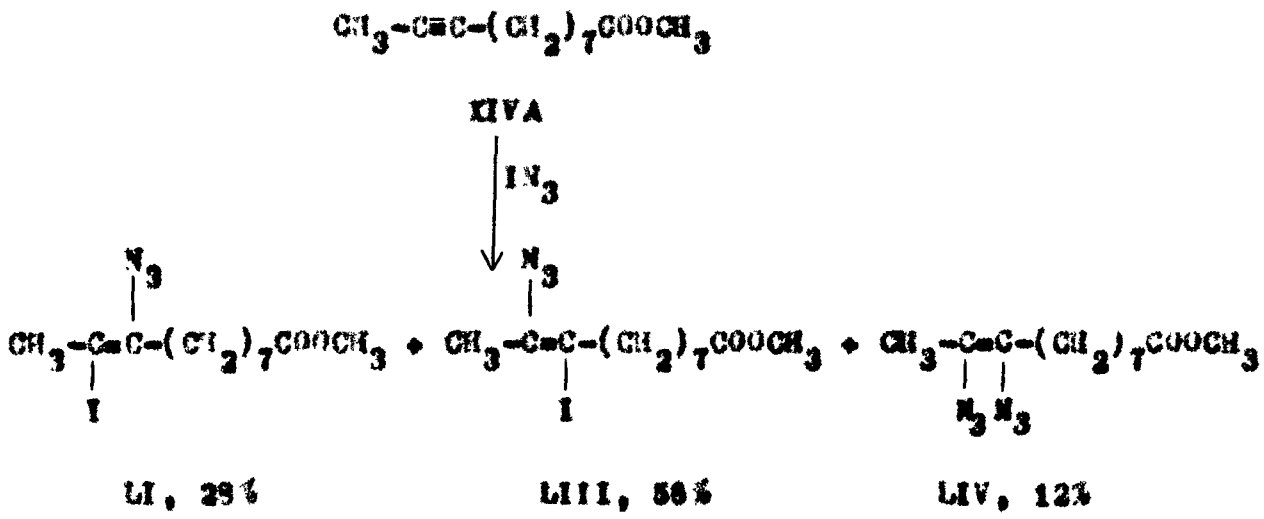
Reduction of vinyl iodoazide (XLV)

The reaction of vinyl iodoazide (XLV) with sodium sulphide in methanol in presence of catalytic amount of triethylamine (TEA) followed by work-up with aqueous acid (Scheme 30) furnished a solid compound XLVIII, m.p. 56-57°C, which showed no depression on mixed m.p. determination with an authentic sample of 10-oxoundecanoic acid. Its microanalysis supported the formula $C_{11}H_{20}O_3$. The product did not contain any halogen or nitrogen as evidenced by IR and Beilstein test. Its 2:4-dinitrophenylhydrazone derivative had m.p. 115-116°C, and its semicarbazone melted at 136°C. In IR spectrum strong bands at 1725 and 1710 cm^{-1} were observed for ester carbonyl and oxo carbonyl functions, respectively. The NMR spectrum showed the signals at δ 1.35 (12H, broad s, $-CH_2-$), 2.05 (3H, s, $CH_3-\overset{O}{\overset{\|}{C}}-$), 2.26 (4H, t like, $-\overset{O}{\overset{\|}{C}}-CH_2$ and $-CH_2-COO$), and 10.10 (1H, s, $-COOH$, vanished in presence of D_2O). A reasonable pathway for the formation of keto acid (XLVIII) may be similar to one illustrated by Nelinka et al.[117] in facile conversion of vinyl azides to ketones. The reduction of XLV with zinc in aqueous acetic acid[114] was also performed which led in nearly quantitative yield to the formation of the same keto acid (XLVIII). The exclusive formation of 10-oxoundecanoic acid (XLVIII) by the reduction of IN_3 adduct (XLV) proves the regiospecificity of XLV and marks the position of azido function at C-10. Hence it establishes the regioselectivity of IN_3 addition to the terminal acetylenic acid.

5.2. Addition of IN_3 to methyl 9-undecynoate (XIVA)

Addition of a pseudohalogen, iodine azide, to methyl 9-undecynoate (XIVA) was carried out in the similar manner (Scheme 47). In this case a separable isomeric mixture of methyl 9(10)-azido-10(9)-iodoundecenoates(LI and LIII) was formed. In addition to these vicinal iodoazides, a diazide (LIV) was also isolated during the reaction which was characterised as 9,10-diazidoundecenoate. After the addition of IN_3 to XIVA, the resulting product was obtained as an amber coloured oil (yield 100%) which showed three distinct spots (R_f = 0.80, 0.70, and 0.36 with respect to the R_f of starting material as 0.77) on an analytical TLC plate. Column chromatography of the crude product revealed LI (28%), LIII (56%), and LIV (12%).

Scheme 47



Characterisation of the addition products

All three products showed a strong band at 2100 cm^{-1} in IR. The elemental analyses of first two isomeric products (LI and LIII) separated from column revealed the molecular formulae as $C_{12}H_{20}O_2IN_3$. Presence of iodo and azido groups was inferred from Beilstein test and nitrogen analysis, respectively. The IR spectra of both were very much alike due to identical groupings. The spectra showed characteristic bands at 2100s ($C-N_3$ str), 1735s (C=O str), 1620m (C=C str), and 720s cm^{-1} (C-I str). The final structure elucidation was made by NMR and mass spectral studies.

The NMR spectrum of LI (Figure 11) gave signals at δ 1.39 (10H, broad s, $-CH_2-$), 2.25 (2H, t like, $-CH_2-COO$), 2.38 (2H, t, $=C(N_3)-CH_2-$), 2.60 (3H, s, $CH_3-C(I)=$), and 3.80 (3H, s, $-COOCH_3$). The mass spectrum (Figure 12) showed peaks among others at m/e 365 (parent peak, $C_{12}H_{20}O_2IN_3$), 323, 210, 208, 205, 193, 154, and 27. The fissions of the molecule leading to fragments can be rationalised as shown in Scheme 48.

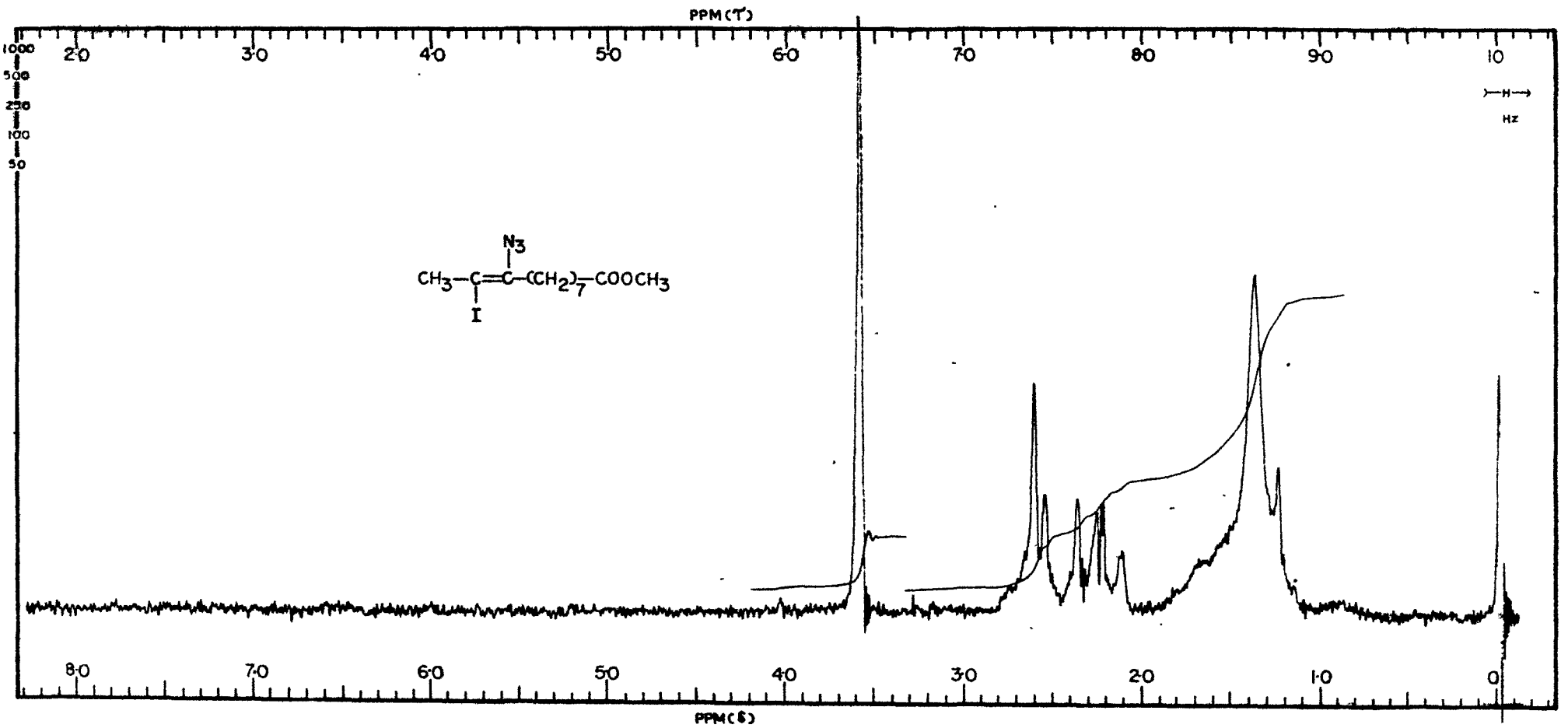


Fig. II. NMR Spectrum of Methyl 9-azido-10-iodoundecenoate (L1)

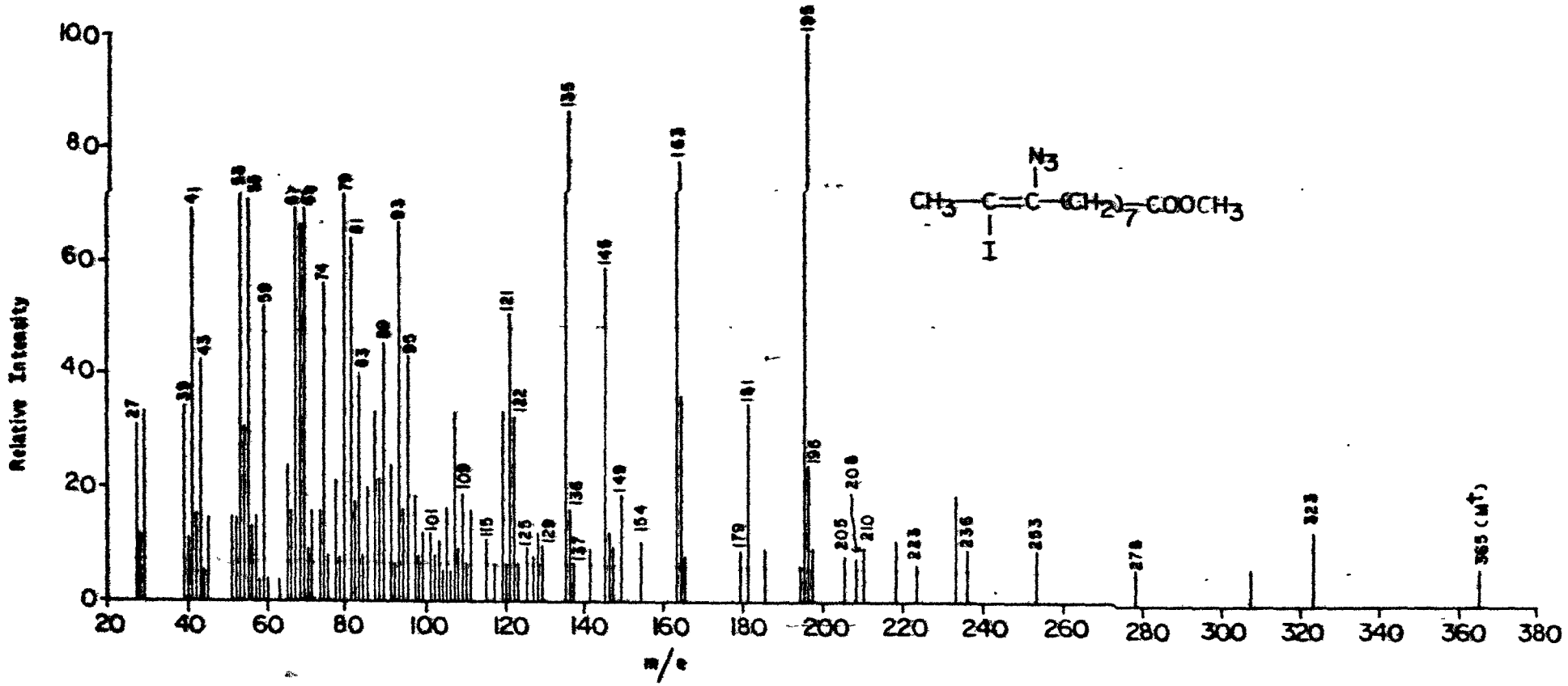
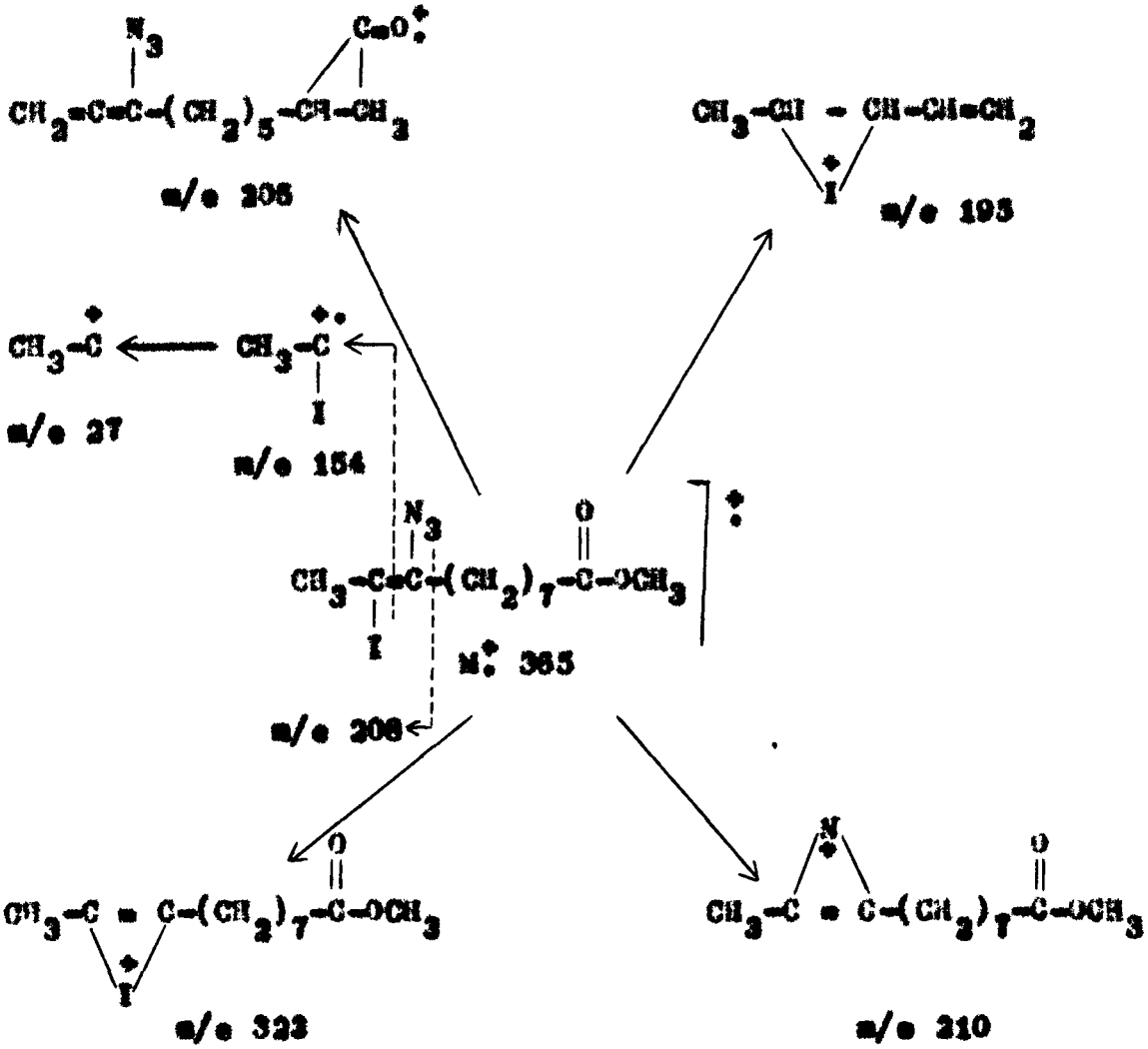


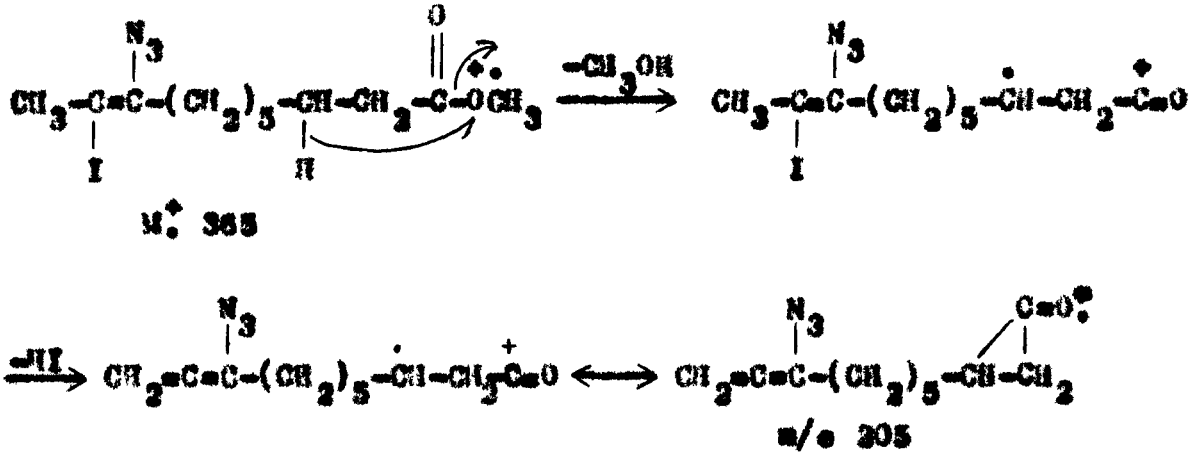
Fig. 12. Mass Spectrum of Methyl 9- azide-10-iodoundecenoate (L1)

Scheme 48



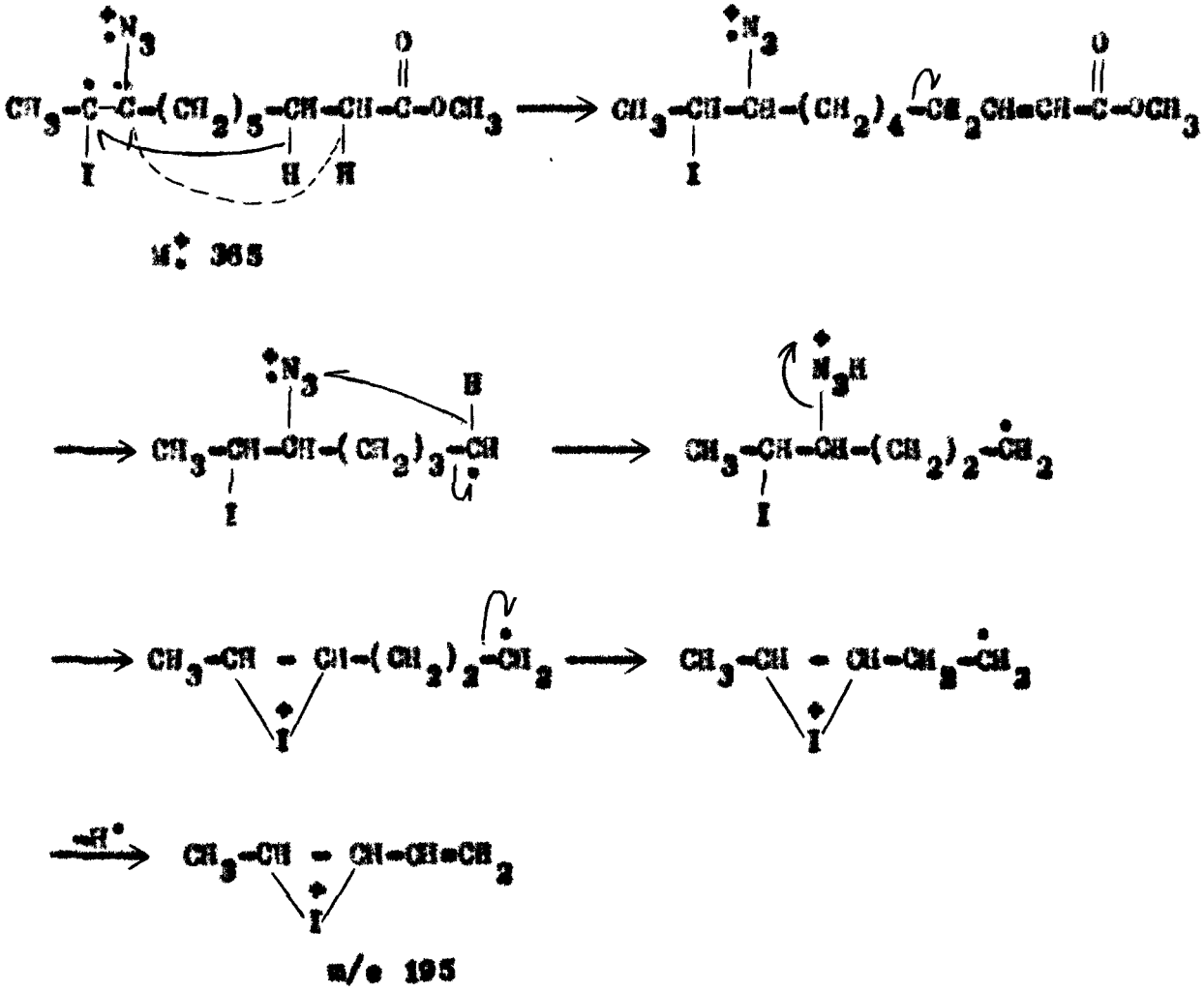
The formation of the fragment ion m/e 205 can be shown according to the mechanism given in Scheme 49.

Scheme 49



The ion peak m/e 195 constitutes the base peak of the spectrum. The corresponding ion could possibly be obtained from the molecular ion according to Scheme 50.

Scheme 50



m/e 195

The above spectral data account well for the structure of LI as methyl 9-azide-10-iodoundecenoate.

The NMR spectrum of LIII (Figure 13) showed signals at δ 1.39 (10H, broad s, $-CH_2-$), 2.10 (3H, s, CH_3-CN_3), 2.08-2.50

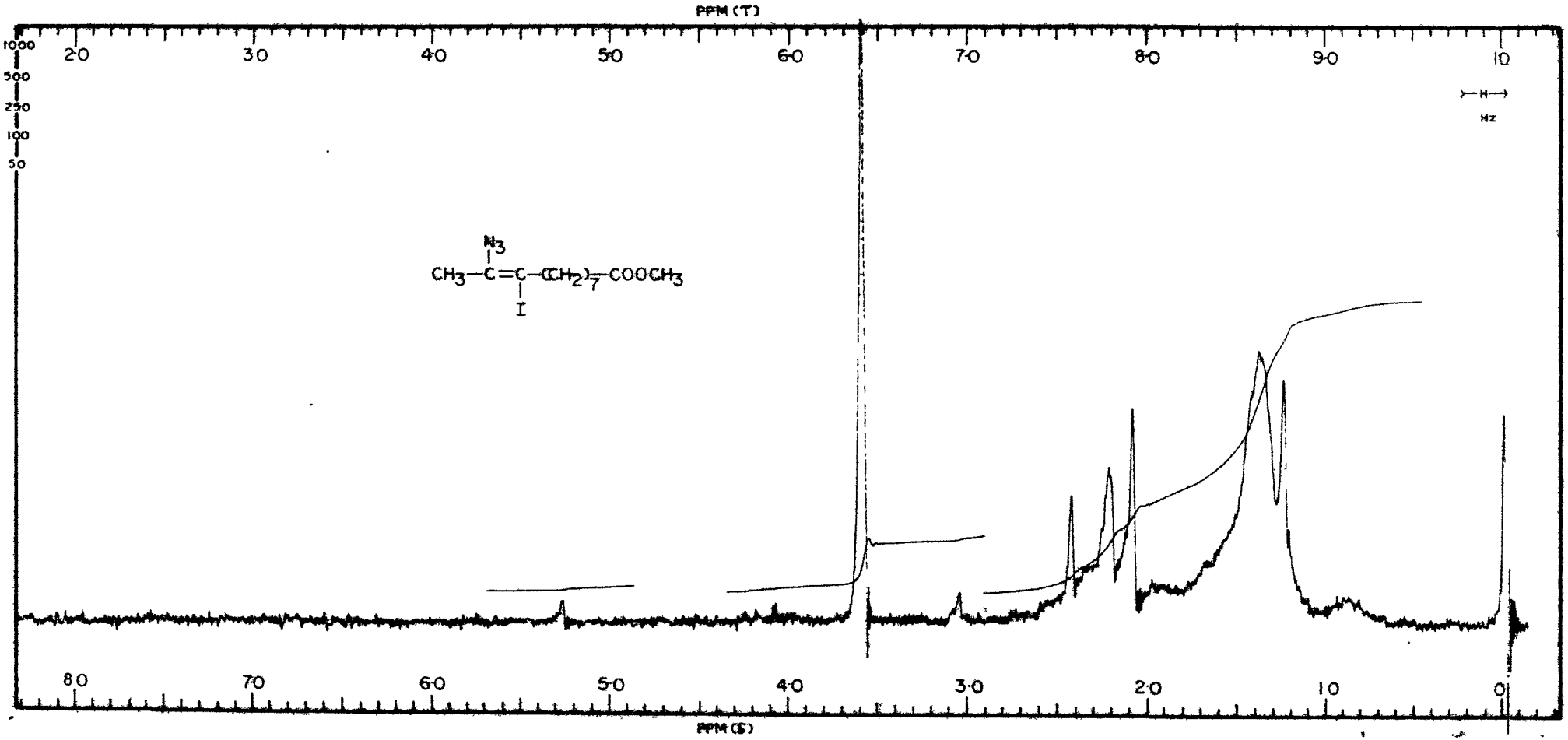


Fig. 13. NMR Spectrum of Methyl 10-azido-9-iodoundecenoate (LIII)

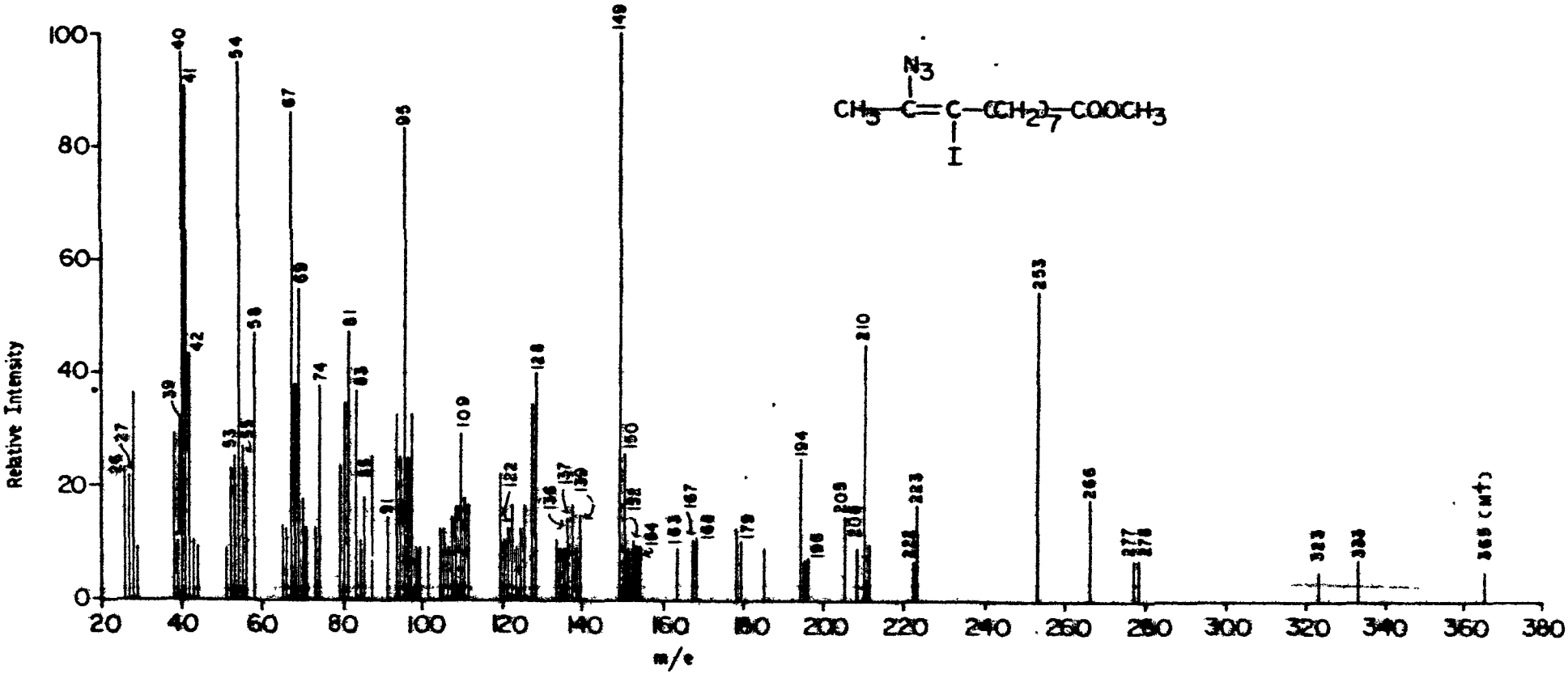
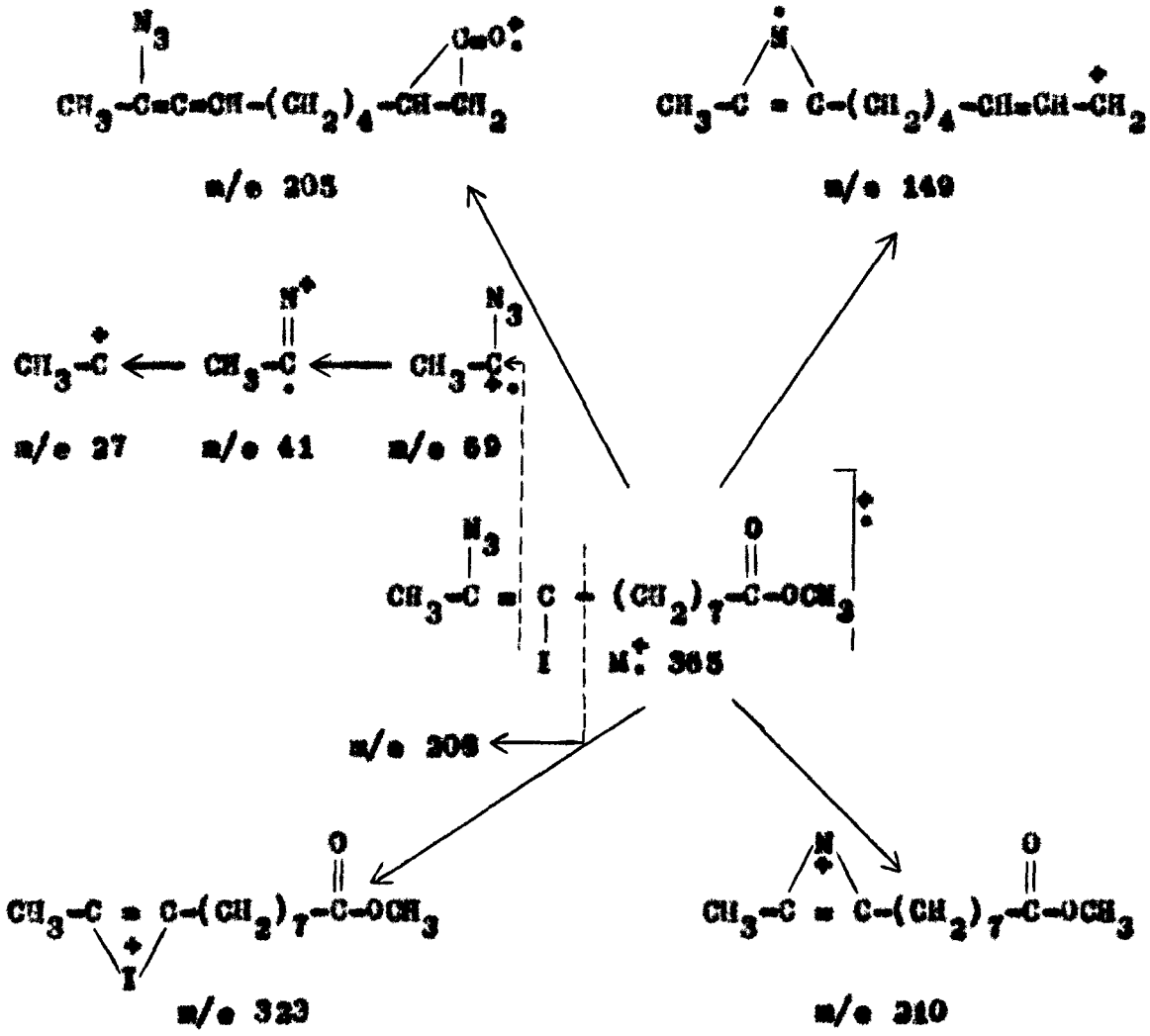


Fig. 14. Mass Spectrum of Methyl 10-azido-9-iodooctadecanoate (LIII)

(4H, overlapping unresolved signals, $\equiv C-\underset{I}{CH_2}-$ and $-\underline{CH_2}-COO$), and 3.62 (3H, s, $-COO\underline{CH_3}$). The mass spectrum (Figure 14) gave $M^{\cdot+}$ at m/e 365 ($C_{12}H_{20}O_2IN_3$) with the significant peaks among others at m/e 323, 210, 208, 149, 69, 41, and 27. The spectral fragmentation is shown in Scheme 51. The ion peak m/e 149 constitutes

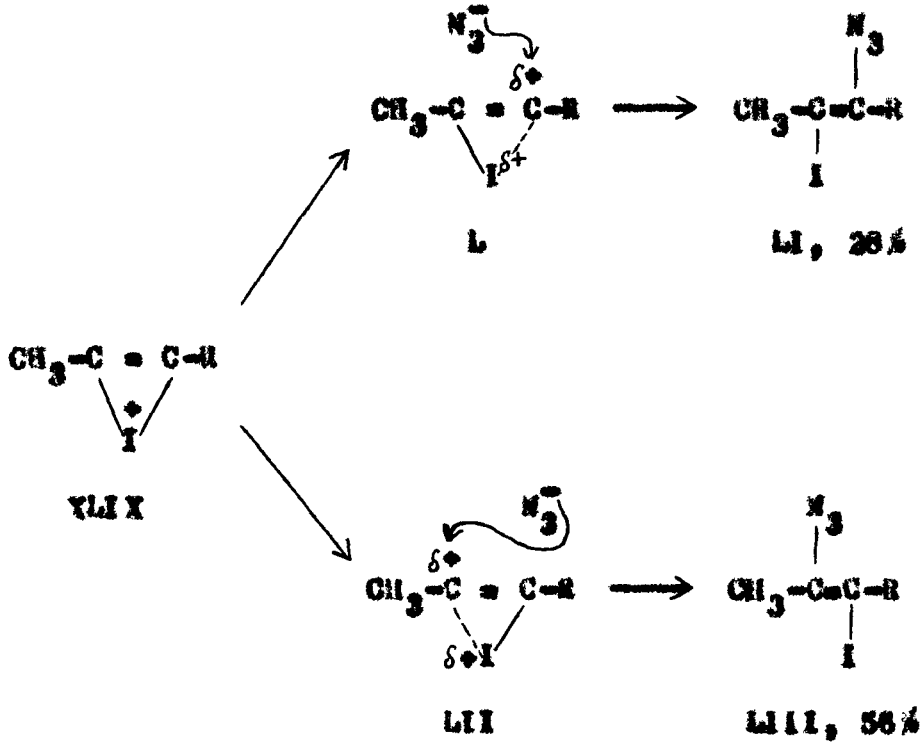
Scheme 51



the base peak of the spectrum. The mechanisms for the formation of the fragment ions m/e 205 and 149 are similar to those described

in Scheme 51 and 45, respectively. The aforesaid data assigned the structure of LIII as methyl 10-azido-9-iodoundecenoate.

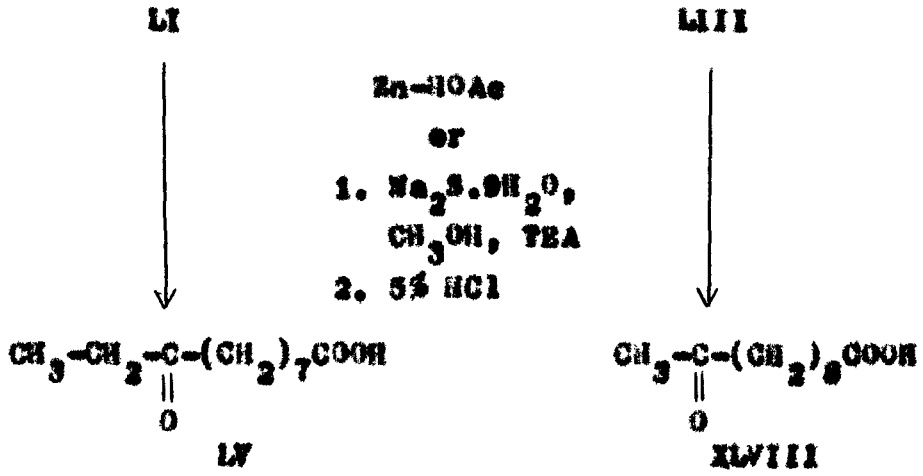
The formation of 1:2 mixture of LI:LIII by the addition of IN_3 to penultimate acetylenic ester (XIVA) can be shown according to the mechanism which involves the cyclic iodonium ion (XLIX) intermediate.[113,114]



Opening of the iodonium ion (XLIX) by N_3^- preferably from less sterically hindered side (carbon α to methyl) leading to LIII (via LII) as the major product is understandable.

Further the reduction of LI and LIII with zinc in aqueous acetic acid[114] and/or by Na_2S method[117] led to the formation of 9- and 10-ketoundecanoic acid, (LV and XLVIII), respectively.

It proves that the products LI and LIII formed by the addition of HN_3 to methyl 9-undecynoate are regioisomers and marks the



position of azide group at C-9 in LI and at C-10 in LIII. The product (m.p. 53°C) LV responded to DNP test.[98] Elemental analysis corresponded to $C_{11}H_{20}O_3$. Its 2:4-dinitrophenylhydrazone and semicarbazone derivatives melted at 138-39° and 160°C, respectively. The IR spectrum showed a double carbonyl band at 1720 (acid C=O str) and 1710 cm^{-1} (oxo C=O str). The NMR spectrum of the keto acid was more informative about its structure. It gave signals at δ 1.12 (3H, s, $\underline{CH}_3$-C-), 1.34 (10H, broad s, -$\underline{CH}_2$-), 2.33 (6H, m, -$\underline{CH}_2$-C(=O)-$\underline{CH}_2$- and -$\underline{CH}_2$-COO), and 10.10 (1H, s, -COO$\underline{H}$, disappeared upon addition of D_2O). The methylene protons α to carboxyl and keto groups appeared at relatively low field because of their association with the deshielding carbonyl groups. Thus the appearance of a six proton multiplet centered at δ 2.35 and the absence of any other signal between δ 1.40 and 2.20 clearly established the keto group at C-9 of the fatty acid chain.

The third product LIV from IN_3 addition to XIVA was characterised as methyl 9,10-diazidoundecenoate. From elemental analysis the formula $C_{12}H_{20}O_2N_6$ was well documented. Presence of azido group and absence of iodo group were also established by positive test for nitrogen and a negative Beilstein test. Characteristic IR bands at 2100s (C-N_3 str) and 1735s cm^{-1} (C=O str) were evidences in favour of the structure. The NMR spectrum showed signals at δ 1.40 (10H, broad s, $-\underline{CH}_2-$), 2.09-2.50 (overlapping signals; 3H, s, $\underline{CH}_3-\underset{N_3}{C}=$; and 4H, t, $=\underset{N_3}{C}-\underline{CH}_2-$ and $-\underline{CH}_2-COO$), and 3.62 (3H, s, $-COO\underline{CH}_3$). The chemical shift of C-8 and C-11 protons which appeared in the area δ 2.09-2.50 indicated the presence of double bond between carbon atoms bearing azide groups. The mass spectrum of LIV (Figure 15) gave peaks among others at m/e 280 (M^+, $C_{12}H_{20}O_2N_6$), 211, 183, 169, 123, 69, 41, and 27. These peaks attributed to the fragments illustrated in Scheme 52. The distinctive feature is the fragment m/e 123, which proved the double bond between C-9 and C-10.

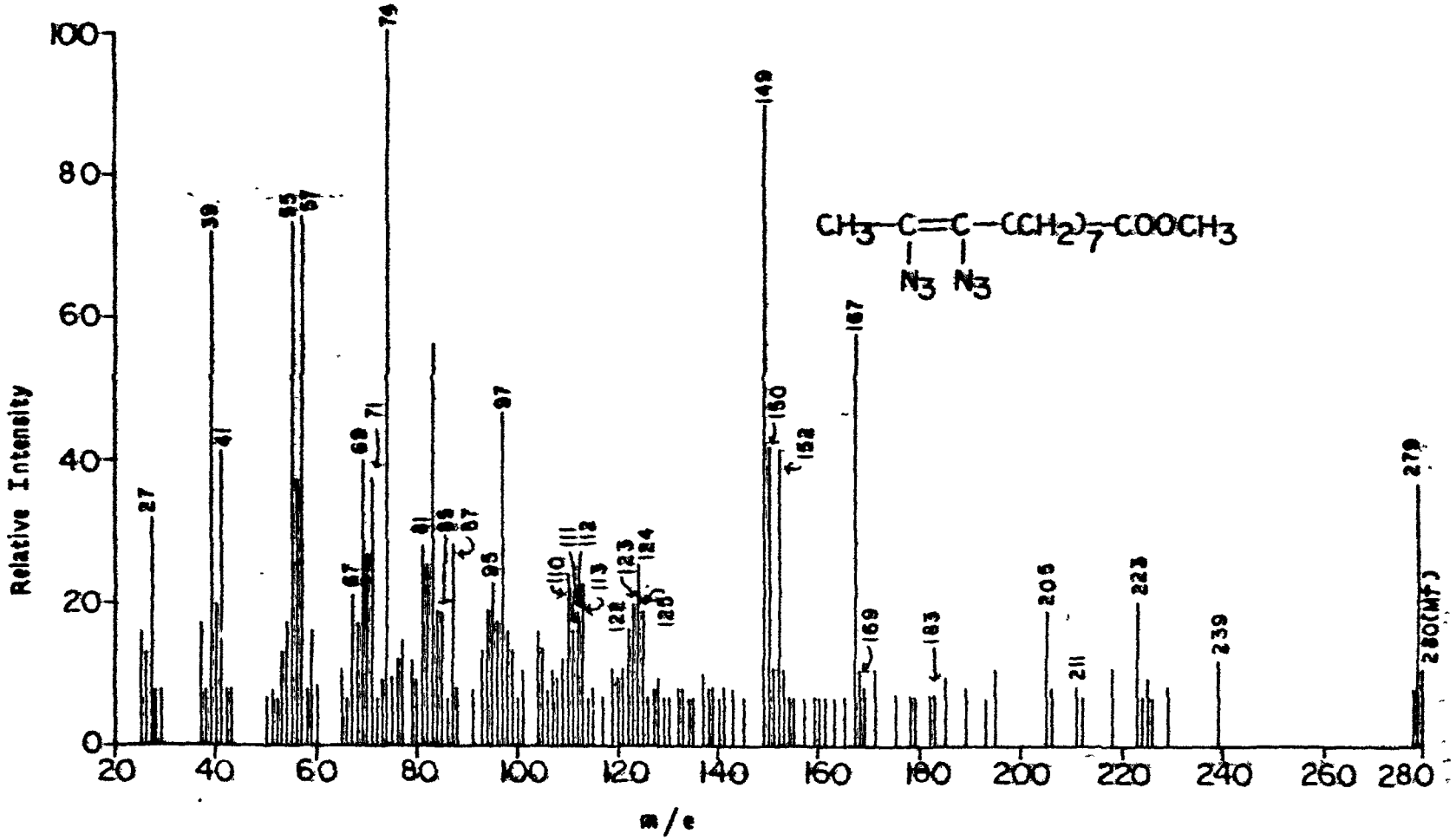
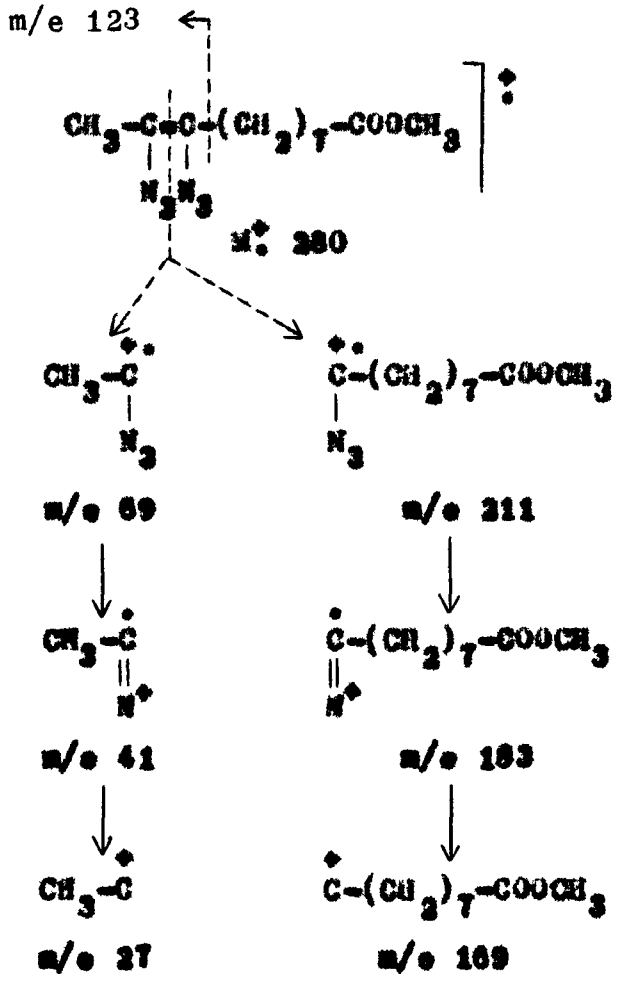


Fig 15. Mass spectrum of Methyl 9,10-diazidoundecenoate (LIV)

Scheme 52



The diazide (LIV, 12%) was isolated as a by-product in the addition reaction. It was shown that this product is also formed upon treatment of XIVA with iodine azide solution at 30°C and therefore probably arises by displacement of the vinylic iodide with azide. Hassner *et al.*[114] has reported the similar reaction product of IN_3 addition to 1-phenylpropyne.

EXPERIMENTAL PROCEDURES

All melting points were observed on a Kofler apparatus and are uncorrected. Infrared (IR) spectra were obtained with a Perkin-Elmer 621 spectrophotometer. The abbreviations "s, m, w, and b" denote "strong, medium, weak, and broad", respectively. Ultraviolet (UV) spectra were determined with a Beckman DK-2A spectrophotometer. Nuclear Magnetic Resonance (NMR) spectra were obtained on a Varian A-60D spectrometer. Chemical shifts are reported as δ (ppm) relative to tetramethylsilane (TMS). The samples were run as 10% solution in carbon tetrachloride. The abbreviations "s, d, t, q, and m" denote "singlet, doublet, triplet, quartet, and multiplet", respectively. Mass spectra were recorded on a Hitachi RMS-4 mass spectrometer. Samples were introduced by direct insertion or via a Varian 1868-4 chromatograph. Spectra were normally run at 70 ev with a source pressure of 10^{-6} torr and a temperature of 150-180°C. Thin-layer chromatographic (TLC) plates were coated with silica gel G, and a mixture of petroleum ether-ethyl ether-acetic acid (80:20:1,v/v/v) was used as developing solvents. Analytical plates were rendered visual by spraying with 20% aqueous solution of perchloric acid and heating in an oven (~110°C) for 10 min. Petroleum ether refers to a fraction of b.p. 40-60°C.

Preparation of 10-undecynoic acid (XII)

To a cold solution of commercial 10-undecenoic acid (X, 40 g) in carbon tetrachloride (150 ml), bromine (25 g) was added dropwise. When the addition was over, the mixture was stirred for 3 hr and left overnight. Distillation of carbon tetrachloride, and working-up with ethyl ether furnished 10,11-dibromoundecanoic acid (XI, 75 g) as a thick viscous liquid. Dibromide (XI, 37.5 g), potassium hydroxide (75 g), water (20 ml), and ethanol (315 ml) were taken in a round

bottomed flask and refluxed on a water-bath for 10 hr. Most of the alcohol was removed under reduced pressure. The product was dissolved in water, acidified with cold dil. H_2SO_4, and extracted with ethyl ether. The extracts were washed with water and dried over anhydrous sodium sulphate. Removal of solvent yielded the crude acid which was crystallised from petroleum ether. The crystalline 10-undecynoic acid (XII, 13.5 g, ca. 55%) thus obtained melted at 42°C (lit.[84] m.p. 41-42°C). Analysis. Found: C, 72.55; H, 9.96. Calc. for $C_{11}H_{18}O_2$: C, 72.53; H, 9.99%.

A portion of the acetylenic acid (XII, 7 g) was esterified with ethereal diazomethane. The corresponding methyl ester (XIIA) was obtained as an oil. Analysis. Found: C, 73.43; H, 10.1%. Calc. for $C_{12}H_{20}O_2$: C, 73.47; H, 10.20%. IR (Neat): 3270s (C-H str), 2110w (C≡C str), and 1740s cm^{-1} (ester C=O str). NMR: δ 1.32 (12H, broad s, $-(CH_2)_2-$), 1.80 (1H, t like, $HC{\equiv}C-$), 1.98-2.40 (4H, overlapping signals, $-C{\equiv}C-CH_2-$ and $-CH_2-COO$), and 3.60 (3H, s, $-COOCH_3$). Mass: m/e 196 (2.7, M^+), 165 (17.3), 164 (10.7), 137 (4.7), 123 (14.7), 122 (15.3), 109 (12.7), 95 (48.7), 83 (28.0), 81 (100), 74 (58.0), 69 (42.0), 67 (69.3), 55 (92.0), 53 (17.3), 43 (44.0), 41 (72.0), 40 (5.3), and 39 (22.0).

Preparation of 9,10-undecadienoic (XIII) and 9-undecynoic (XIV) acids

Another portion of 10,11-dibromoundecanoic acid (XI, 37.5 g) was added to a solution of potassium hydroxide (56.5 g) in water (35 ml) and the mixture was heated in an open flask so that the internal temperature rose slowly to 180°C and remained there for 30 min. Water (170 ml) was added and the solution acidified with dil. H_2SO_4, the product being extracted with ethyl acetate. Evaporation of the solvent gave the product (18.75 g) as a non-crystallisable oil which showed two very close spots on TLC plate.

The crude product was chromatographed over a column of silica gel. Elution with petroleum ether gave 9,10-undecadienoic acid (XIII, 3.75 g, ca. 15%) as an oil (each fraction of 25 ml was collected). It resisted all attempts to crystallisation. Analysis. Found: C, 72.49; H, 9.87. Calc. for $C_{11}H_{18}O_2$: C, 72.53; H, 9.89%. A portion of the allenic acid (XIII, 1 g) was esterified with ethereal diazomethane to yield methyl 9,10-undecadienoate (XIIIA). Analysis. Found: C, 73.52; H, 10.23. Calc. for $C_{12}H_{20}O_2$: C, 73.47; H, 10.20%. IR (Neat): 1950s, 1710w and 850s (C=C=C str), and 1740s cm^{-1} (ester C=O str). NMR: δ 1.32 (10H, broad, s, $-CH_2-$), 2.05-2.50 (4H, overlapping signals, $>C=C=C-CH_2-$ and $-CH_2-COO$), 3.62 (3H, s, $-COOCH_3$), 5.35 and 5.55 (2H, both m, $\underline{H}\diagdown\ \ \diagup\underline{H}$ $C=C=C-$), and 6.06

(1H, m, >C=C-C<u>H</u>-). Mass: m/e 197 (19.3), 196 (36.7 $M^{\cdot}$), 165 (50.3), 164 (37.3), 137 (14.0), 123 (50.0), 122 (32.0), 109 (19.3), 97 (32.0), 95 (53.3), 83 (46.7), 81 (100), 69 (58.7), 67 (71.3), 55 (92.7), 53 (42.7), 43 (55.3), 41 (91.3), and 39 (55.3).

Subsequent elution with a mixture of petroleum ether-ethyl ether (98:2, v/v) gave 9-undecynoic acid (XIV, 11.25 g, ca. 45%) as a white crystalline solid, m.p. 59°C (lit.[85] m.p. 58-59°C). <u>Analysis</u>. Found: C, 72.43; H, 9.80. Calc. for $C_{11}H_{18}O_2$: C, 72.53; H, 9.89%. A portion of the acid (XIV, 7 g) was esterified with ethereal diazomethane. The resulting methyl 9-undecynoate (XIVA) was obtained as an oil. <u>Analysis</u>. Found: C, 73.41; H, 10.18. Calc. for $C_{12}H_{20}O_2$: C, 73.47; H, 10.20%. IR (Neat): 2210w (C≡C str) and 1740s cm^{-1} (ester C=O str.). NMR: δ 1.35 (10H, broad s, -C<u>H</u>$_2$-), 1.74 (3H, t like, C<u>H</u>$_3$-C=C-), 2.0-2.50 (4H, overlapping signals, -C≡C-C<u>H</u>$_2$- and -C<u>H</u>$_2$-COO), and 3.65 (3H, s, -COOC<u>H</u>$_3$). Mass: m/e 197 (4.0), 181 (4.0), 165 (4.7), 149 (4.0), 137 (7.3), 123 (13.3), 122 (9.3), 109 (18.0), 107 (23.3), 99 (20.7), 97 (42.7), 95 (32.0), 83 (47.3), 81 (42.7), 71 (69.3), 69 (55.3), 67 (34.7), 57 (100), 55 (59.7), 54 (14.0), 53 (12.0), 41 (42.0), and 39 (12.0).

Perbenzoic acid oxidation of methyl 10-undecynoate (XIIA)

To the solution of methyl 10-undecynoate (XIIA, 2 g, 0.010 mole) in chloroform (35 ml), freshly prepared perbenzoic acid solution[cf. 99a] (51.6 ml; strength 4.5%, 0.020 mole) was added with shaking. The mixture was kept at room temperature for 8 days and examined by direct and picric acid[97] TLC. After 24 hr the reaction mixture gave faint orange spot in picric acid TLC indicating the formation of oxirene and showed gradual disappearance of the spot after 3 days. The chloroform was evaporated in vacuo under the stream of nitrogen and the resulting mixture was taken up in ethyl ether. This was washed with 5% solution of sodium bicarbonate and then with water till the ethereal layer becomes neutral, and dried over anhydrous sodium sulphate. Evaporation of the solvent gave the product as a viscous oil which showed four distinct TLC spots with negative picric acid TLC test.

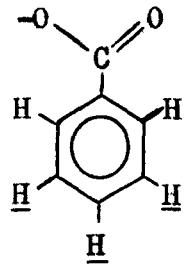
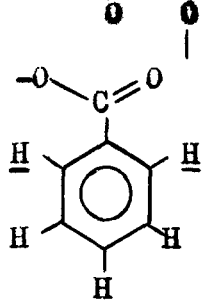
The crude product (2.8 g) was chromatographed over a column of silica gel (55 g) and the elution was carried out with petroleum ether containing increasing amounts of ethyl ether.

Methyl 10-undecynoate (XIIA)

Elution with petroleum ether gave unreacted starting material (XIIA, 1.26 g, ca. 45%) (each fraction of 25 ml was

collected). Analysis. Found: C, 73.80; H, 10.22. Calc. for $C_{12}H_{20}O_2$: C, 73.47; H, 10.20%. IR (Neat): 3270s (C-H str), 2110w (C≡C str), and 1740s cm^{-1} (ester C=O str). NMR: δ 1.34 (12H, broad s, $-\underline{CH}_2-$), 1.82 (1H, t like, $\underline{H}C\equiv C-$), 2.0-2.42 (4 H, overlapping signals, $-C\equiv C-\underline{CH}_2$ and $-\underline{CH}_2-COO$), and 3.62 (3H, s, $-COOC\underline{H}_3$).

Methyl 11-aldehyde-10-benzoyloxyundecanoate (XXII)

Subsequent elution with a mixture of petroleum ether-ethyl ether (98:2, v/v) gave XXII (0.45 g, ca. 16%) as an oil. Analysis. Found: C, 68.19; H, 7.59. Calc. for $C_{19}H_{26}O_5$: C, 68.26; H, 7.78%. IR (Neat): 1740-1720s,b (ester C=O and aldehyde C=O str), 1690s (aryl C=O str), and 1610m cm^{-1} (aromatic C=C str). NMR: δ 1.22 (14H, broad s, $-\underline{CH}_2-$), 2.20 (2H, t, $-\underline{CH}_2-COO$), 3.55 (3H, s, $-COOC\underline{H}_3$), 4.16 (1H, m, $-\underset{\underset{O}{\|}}{C}-\underline{CH}(\underline{O}-)-$), 7.30 (3H, m, ), 7.90 (2H, d of d ), and 9.25 (1H, d, $\underline{H}\underset{\underset{O}{\|}}{C}-$).

Half methyl ester of 10-ethoxyundecane-11,1-dioic acid (XXIII)

Further elution with a mixture of petroleum ether-ethyl ether (92:8, v/v) gave XXIII (0.28 g, ca. 10%) as an oil. Analysis. Found: C, 61.36; H, 9.61. Calc. for $C_{14}H_{26}O_5$: C, 61.31; H, 9.49%. IR (Neat): 1740s (ester C=O str), 1710s (acid C=O str), and 1610m and 1090m cm^{-1} (C-O-C). NMR: δ 0.90 (3H, distorted t, $-OCH_2\underline{CH}_3$), 1.20 (14H, broad s, $-\underline{CH}_2-$), 2.20 (2H, t, $-\underline{CH}_2-COO$), 3.55 (3H, s, $-COO\underline{CH}_3$), 3.79 (2H, t, $OOC-\underline{CH}(O-)$), 4.08 (2H, t, $-O\underline{CH}_2CH_3$), and 10.10 (1H, s, $\underline{H}OOC$, vanished in presence of D_2O).

Ethyl, methyl undecane-11,1-dioate (XXIV)

Final elution with ethyl ether gave XXIV (0.30 g, ca. 21%) as an oil. Analysis. Found: C, 65.19; H, 10.03. Calc. for $C_{14}H_{26}O_4$: C, 65.13; H, 10.08%. IR (Neat): 1750-1735s cm^{-1} (ethyl ester C=O and methyl ester C=O str). NMR: δ 0.84 (3H, distorted t, $\underline{CH}_3CH_2OOC-$), 1.25 (14H, broad s, $-\underline{CH}_2-$), 2.25 (4H, t like, $C_2H_5OOC-\underline{CH}_2-$ and $-\underline{CH}_2-COOCH_3$), 3.68 (3H, s, $-COO\underline{CH}_3$), and 4.24 (2H, t, $CH_3\underline{CH}_2OOC-$).

Perbenzoic acid oxidation of methyl 9-undecynoate (XIVA)

Similar treatment (as described in peroxyacid oxidation of XIIA) of methyl 9-undecynoate (XIVA, 2 g, 0.010 mole) in chloroform (25 ml) with freshly prepared perbenzoic acid

solution[cf.99a] (61.6 ml; strength 4.5%, 0.020 mole) yielded an oily product after usual work-up. Picric acid TLC[97] of the reaction mixture showed faint orange spot in the initial stage. The resulting oily product gave five distinct spots in direct TLC and no spot responded to picric acid test.

The crude product (2.58 g) was fractionated by a column of silica gel (50 g) prepared in petroleum ether and eluted with petroleum ether containing increasing amounts of ethyl ether.

Methyl 9-undecynoate (XIVA)

Elution with petroleum ether gave unreacted acetylenic ester (XIVA, 1.01 g, ca. 30%). Analysis. Found: C, 73.46; H, 10.18. Calc. for $C_{12}H_{20}O_2$: C, 73.47; H, 10.20%. IR (Neat): 2210w (C≡C str) and 1740s cm^{-1} (ester C=O str). NMR: δ 1.35 (10H, broad s, $-CH_2-$), 1.74 (3H, t like, $CH_3-C\equiv C-$), 2.0-2.50 (4H, overlapping signals, $-C\equiv C-CH_2-$ and $-CH_2-COO$), and 3.65 (3H, s, $-COOCH_3$).

Half methyl ester of 9-methoxy-9-methyldecane-10,1-dioic acid (XXXII)

Subsequent elution with a mixture of petroleum ether-ethyl ether (99.5:0.5, v/v) gave XXXII (0.13 g, ca. 5%) as an oil. Analysis. Found: C, 59.90; H, 9.03. Calc. for $C_{13}H_{24}O_5$: C, 60.00; H, 9.23%. IR (Neat): 1740-1720s,b (ester C=O and acid C=O str), and 1170m and 1080m cm^{-1} (C-O-C). NMR: δ 1.27

Analysis. Found: C, 68.01; H, 9.58. Calc. for $C_{12}H_{20}O_3$: C, 67.92; H, 9.43%. IR (Neat): 1740s (ester C=O str), 1720s (exo C=O str), and 1620w cm^{-1} (cis C=C str). NMR: δ 1.28 (10H, broad s, $-CH_2-$), 2.21 (3H, s, $CH_3-\overset{\overset{O}{\|}}{C}-$), 2.27 (2H, t, $-CH_2-COO$), 3.65 (3H, s, $-COOCH_3$), 6.0 (1H, d, $-\overset{\overset{O}{\|}}{C}-CH=CH$), and 6.78 (1H, m, $-\overset{\overset{O}{\|}}{C}-CH=CH-$).

Hypobromination of 10-undecynoic acid (XII)

10-Undecynoic acid (XII, 2 g, 0.011 mole) was taken in aqueous potassium hydroxide (100 ml, 0.1N). Then few ml of ethanol was added to form the soap completely. On cooling freshly prepared sodium hypobromite solution (100 ml, 0.5M) was added and the reaction mixture was kept overnight at room temperature. The solution was acidified with dil. sulphuric acid and extracted with ethyl ether. The extracts were washed with sodium sulphite solution (to destroy any excess of bromine) and then with water, and dried over anhydrous sodium sulphate. Removal of the solvent yielded a brown syrupy liquid which showed three distinct spots on an analytical TLC plate. The crude acid was methylated by heating under reflux for 5 hr with anhydrous methanol in the presence of acid catalyst (H_2SO_4).

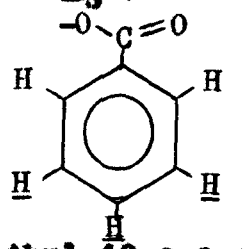
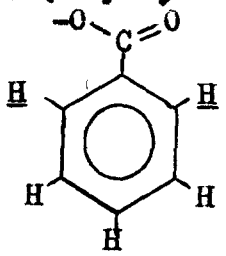
A column of silica gel (80 g), prepared in petroleum ether, was charged with total crude mixture (3.8 g) and eluted with petroleum ether containing increasing amounts of ethyl ether.

(12H, broad s, $-CH_2-$), 1.71 (3H, s, $OOC-\overset{O}{\overset{|}{C}}-CH_3$), 2.28 (2H, t, $-CH_2COO$), 3.68 (3H, s, $-COOCH_3$), 3.78 (3H, s, $OOC-\overset{OCH_3}{\overset{|}{C}}-$), and 10.10 (1H, s, $-COOH$, disappeared upon addition of D_2O).

Unidentified(XXXIII)

Further elution with a mixture of petroleum ether-ethyl ether (98:2, v/v) afforded XXXIII (0.33 g, ca. 9%) as an oil which could not be characterised.

Methyl 8-ethylcarboxy(1'-benzoyloxy)octanoate (XXXIV)

Subsequent elution with a mixture of petroleum ether-ethyl ether (93:7, v/v) gave XXXIV (0.23 g, ca. 9%) as a viscous oil. Analysis. Found: C, 65.04; H, 7.30. Calc. for $C_{19}H_{26}O_6$: C, 65.14; H, 7.43%. IR (Neat): 1740-1700s,b (ethylcarboxy C=O, ester C=O, and aryl C=O), and 1600m and 1580m cm^{-1} (aromatic C=C str). NMR: δ 1.24 (10H, broad s, $-CH_2-$), 1.48 (3H, d, $-O-\overset{CH_3}{\overset{|}{C}}-O-$), 2.30 (4H, m, $-O-\overset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2COO$), 3.67 (3H, s, $-COOCH_3$), 5.32 (1H, q, $-O-\overset{H}{\overset{|}{C}}-O-$), 7.51 (3H, m, ), and 8.60 (2H, d of d, ).

Methyl 10-oxo-cis-8-undecenoate (XXXV)

Final elution with ethyl ether afforded XXXV (0.60 g, ca. 31%)(positive DNP test[98] for the presence of keto group).

Methyl 11-aldehyde-10,10-dibromoundecanoate (XXVI)

Elution with petroleum ether afforded XXVI (1.33 g, ca. 35%) as an oil (positive Beilstein test[99b] for the presence of bromine). Analysis. Found: C, 38.89; H, 5.23; Br, 42.92. Calc. for $C_{12}H_{20}O_3Br_2$: C, 38.71; H, 5.38; Br, 43.01%. IR (Neat): 1740s (ester C=O str), 1730s (aldehyde C=O str), and 720s cm^{-1} (C-Br str). NMR: δ 1.36 (12H, broad s, $-CH_2-$), 2.23 (2H, t like, $-CH_2-COO$), 2.68 (2H, t, $-\underset{Br}{\overset{Br}{C}}-CH_2-$), 3.61 (3H, s, $-COOCH_3$), and 9.30 (1H, s, $\underset{O}{\overset{\|}{HC}}-$).

Methyl 11,11-dibromo-10-oxoundecanoate (XXVII)

Elution with the solvent system petroleum ether-ethyl ether (95:5, v/v) gave XXVII (1.99 g, ca. 52%) as an oil (positive Beilstein[99b] and DNP[98] tests for the presence of bromine and keto functions, respectively). Analysis. Found: C, 38.75; H, 5.47; Br, 43.19. Calc. for $C_{12}H_{20}O_3Br_2$: C, 38.71; H, 5.38; Br, 43.01%. IR (Neat): 1735s (ester C=O str), 1720s (oxo C=O str), and 720s cm^{-1} (C-Br str). NMR: δ 1.36 (12H, broad s, $-CH_2-$), 2.24 (4H, t, $-\underset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2-COO$), 3.62 (3H, s, $-COOCH_3$), and 6.09 (1H, s, $\underset{Br}{\overset{Br}{HC}}-$).

Methyl 9,11-dibromo-10-oxoundecanoate (XXXVIII)

Subsequent elution with ethyl ether gave XXXVIII (0.37 g, ca. 10%) as an oil (positive Beilstein and DNP tests for the presence of bromine and keto functions, respectively). Analysis. Found: C, 38.57; H, 5.23; Br, 43.24. Calc. for $C_{12}H_{20}O_3Br_2$: C, 38.71; H, 5.38; Br, 43.01%. IR (Neat): 1750s (ester C=O str), 1725s (oxo C=O str), and 720s cm^{-1} (C-Br str). PMR: δ 1.32 (12H, broad s, $-CH_2-$), 2.26 (2H, t, $-CH_2-COO$), 3.66 (3H, s, $-COOCH_3$), 3.81 (2H, s, $\underset{Br}{\underset{|}{H_2C}}-$), and 4.32 (1H, m, $-\underset{Br}{\underset{|}{CH}}-$).

Hypobromination of 9-undecynoic acid (XIV)

Similarly, the potassium salt of 9-undecynoic acid (XIV, 2 g, 0.011 mole) and sodium hypobromite (100 ml, 0.5M) furnished a brown syrupy liquid showing three distinct spots on TLC plate.

After esterification, the crude mixture (3.9 g) was chromatographed over a column of silica gel (80 g) (packed in petroleum ether) and elution was carried out with a mixture of petroleum ether-ethyl ether.

Methyl 10-bromo-9-oxoundecanoate (XL)

Elution with a mixture of petroleum ether-ethyl ether (99:2, v/v) gave XL (1.84 g, ca. 47%) as an oil (positive Beilstein and DNP tests for the presence of bromo and keto

groups, respectively). Analysis. Found: C, 40.21; H, 7.05; Br, 37.21. Calc. for $C_{12}H_{21}O_3Br$: C, 40.18; H, 7.17; Br, 37.30%. IR (Neat): 1748s (ester C=O str), 1720s (exo C=O str), and 720s cm^{-1} (C-Br str). NMR: δ 1.31 (10H, broad s, $-CH_2-$), 2.23 (4H, t, $-\overset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2-COO$), 2.61 (2H, d, $CH_2-\underset{Br}{\overset{|}{C}}-$), 3.10 (1H, q, $-\underset{Br}{\overset{|}{CH}}-$), and 3.61 (3H, s, $-COOCH_3$).

Methyl 9,9-dibromo-10-oxoundecanoate (XLI)

Elution with a mixture of petroleum ether–ethyl ether (94:6, v/v) gave XLI (1.45 g, ca. 37%) as an oil (positive Beilstein and DNP tests for the presence of bromo and keto groups, respectively). Analysis. Found: C, 38.64; H, 5.15; Br, 43.08. Calc. for $C_{12}H_{20}O_3Br_2$: C, 38.71; H, 5.29; Br, 43.01%. IR (Neat): 1740s (ester C=O str), 1710s (exo C=O str), and 720s cm^{-1} (C-Br str). NMR: δ 1.30 (10H, broad s, $-CH_2-$), 2.24 (2H, t, $-CH_2-COO$), 2.45 (3H, s, $CH_3-\overset{O}{\overset{\|}{C}}-$), 2.67 (2H, t, $-\underset{Br}{\overset{Br}{\overset{|}{C}}}-CH_2-$), and 3.63 (3H, s, $-COOCH_3$).

Methyl 10,10-dibromo-9-oxoundecanoate (XLII)

Elution with a mixture of petroleum ether–ethyl ether (92:8, v/v) gave XLII (0.46 g, ca. 12%) as an oil (positive Beilstein and DNP tests for the presence of bromo and keto

groups, respectively). Analysis. Found: C, 39.78; H, 5.17; Br, 42.94. Calc. for $C_{12}H_{20}O_3Br_2$: C, 38.71; H, 5.38; Br, 43.01%. IR (Neat): 1740s (ester C=O str), 1725s (oxo C=O str), and 730s cm^{-1} (C-Br str). NMR: δ 1.32 (10H, s, $-CH_2-$), 2.24 (4H, t, $-\overset{\overset{O}{\|}}{C}-CH_2$ and $-CH_2-COO$), 2.51 (3H, s, $CH_3-\overset{\overset{Br}{|}}{\underset{\underset{Br}{|}}{C}}-$), and 3.62 (3H, s, $-COOCH_3$).

Reaction of iodine azide with methyl 10-undecynoate (XIIA) Preparation of methyl 10-azido-11-iodoundecenoate (XLV)

Methyl 10-undecynoate (XIIA) was treated with IN_3 according to the procedure of Fowler et al.[115] To a stirred slurry of sodium azide (6.50 g, 0.10 mole) in acetonitrile (30 ml) in an ice-bath was added slowly iodine monochloride (7.14 g, 0.044 mole) over a period of 20 min. The reaction mixture was stirred for additional 10 min and after the acetylenic ester (XIIA, 4 g, 0.02 mole) was added, allowed to warm to room temperature and again stirred for 12 hr. The red brown slurry was poured into water (100 ml) and the mixture was extracted with ethyl ether (100 ml) in three portions. These were combined and washed with 5% sodium thiosulphate solution (50 ml) leaving a colourless ethereal solution. The solution was again washed with water (500 ml) in four portions and dried over anhydrous sodium sulphate. Removal of ethyl ether in vacuo at room temperature produced the iodoazide adduct (yield ~ 100%), now slightly orange in colour.

The trace of starting material was separated by column chromatography over silica gel and eluted with petroleum ether containing increasing amounts of ethyl ether. Eluted material was monitored by TLC. Elution with petroleum ether–ethyl ether (98:2, v/v) yielded methyl 10-azido-11-iodoundecanoate (XLV) as an amber oil (positive Beilstein test[99b] for the presence of iodine). Analysis. Found: C, 39.53; H, 5.59; N, 11.45. Calc. for $C_{12}H_{20}O_2IN_3$: C, 39.45; H, 5.48; N, 11.51%. IR (Neat): 2920s (C-H str), 2110s (C-N_3 str), 1735s (C=O str), 1610m(C=C str), and 720s cm^{-1} (C-I str). NMR: δ 1.40 (12H, broad, s, $-CH_2-$), 2.26 (4H, t like, $=\overset{N_3}{\overset{|}{C}}-CH_2-$ and $-CH_2-COO$), 3.66 (3H, s, $-COOCH_3$), and 5.32 (1H, s, $\overset{I}{\overset{|}{HC}}=$). Mass: m/e 365 (5.3, $M^{\cdot+}$), 333 (9.3), 305 (6.7), 291 (8.0), 278 (6.7), 277 (6.7), 267 (93.3), 266 (6.7), 253 (6.7), 250 (8.0), 236 (5.3), 223 (9.3), 222 (6.7), 210 (16.0), 209 (14.7), 206 (6.7), 205 (9.3), 198 (8.0), 195 (8.0), 180 (6.7), 178 (10.7), 171 (10.7), 166 (16.0), 150 (16.0), 149 (100), 140 (8.0), 139 (46.7), 137 (6.7), 125 (13.3), 123 (8.0), 122 (8.0), 121 (20.0), 109 (17.3), 97 (44.0), 95 (20.0), 83 (26.7), 81 (26.7), 79 (13.3), 74 (20.0), 69 (49.3), 68 (10.7), 67 (29.3), 55 (69.3), 53 (13.3), 43 (18.7), 41 (68.0), 40 (8.0), and 39 (10.7).

Reaction of iodine azide with methyl 9-undecynoate (XIVA)

Similar addition of IN_3 [prepared in situ from sodium azide (6.50 g, 0.10 mole) and iodine monochloride (7.14 g, 0.044 mole) in acetonitrile (40 ml) solution] to methyl 9-undecynoate (XIVA, 4 g, 0.02 mole) produced the iodoazide adduct (100%) as an amber-coloured oil. The oily product showed three distinct spots in TLC. This was chromatographed over a column of silica gel (40 g) and the elution was carried out with petroleum ether containing increasing amounts of ethyl ether.

Methyl 9-azido-10-iodoundecenoate (LI)

Elution with petroleum ether gave LI (1.12 g, ca. 29%) as an amber oil (positive Beilstein test for the presence of iodine). Analysis. Found: C, 39.39; H, 5.55; N, 11.62. Calc. for $C_{12}H_{20}O_2IN_3$: C, 39.45; H, 5.43; N, 11.51%. IR (Neat): 2100s ($C-N_3$ str), 1735s (C=O str), 1620m (C=C str), and 720s cm^{-1} (C-I str). NMR: δ 1.39 (10H, broad s, $-CH_2-$), 2.25 (2H, t like, $-CH_2-COO$), 2.38 (2H, t, $\equiv C-CH_2-$), 2.60 (3H, s, $CH_3-C=$), and 3.60 (3H, s, $-COOCH_3$). Mass: m/e 365 (6.7, M^+), 323 (13.3), 279 (6.7), 253 (9.3), 238 (9.3), 223 (6.7), 210 (9.3), 208 (8.0), 205 (9.0), 196 (24.0), 195 (100), 181 (34.7), 179 (9.3), 163 (77.3), 154 (10.7), 149 (18.7), 145 (58.7), 137 (6.7), 136 (16.0), 135 (96.7), 129 (9.3), 125 (9.3), 122 (32.0), 121 (50.7), 115 (10.7), 109 (18.7), 101 (12.0), 95 (42.7), 93 (66.7), 99 (45.3), 83 (40.0), 81 (64.0), 79 (72.0), 74 (56.0), 69 (69.3), 68 (66.7), 67 (69.3), 59 (52.0), 55 (70.7), 53 (72.0), 43 (42.7), 41 (69.3), 39 (34.7), and 27 (30.7).

Methyl 10-azido-9-iodoundecanoate (LIII)

Subsequent elution with petroleum ether-ethyl ether (99:1, v/v) gave LIII (2.24 g, ca. 36%) as an amber oil (positive Beilstein test for the presence of iodine). Analysis. Found: C, 39.47; H, 5.45; N, 11.35. Calc. for $C_{12}H_{20}O_2IN_3$: C, 39.45; H, 5.48; N, 11.51%. IR (Neat): 2100s ($C-N_3$ str), 1735s (C=O str), 1620m (C=C str), and 720s cm^{-1} (C-I str). NMR: δ 1.30 (10H, broad s, $-CH_2-$), 2.10 (3H, s, $CH_3-C(N_3)$), 2.00-2.50 (4H, overlapping unresolved signals, $=C(I)-CH_2-$ and $-CH_2-COO$), and 3.62 (3H, s, $-COOCH_3$). Mass: m/e 365 (5.3, $M^{\cdot+}$), 333 (7.3), 329 (8.3), 278 (7.3), 277 (7.3), 264 (16.0), 263 (84.7), 223 (16.7), 222 (7.3), 210 (45.3), 208 (9.3), 205 (14.7), 196 (7.3), 195 (7.3), 194 (25.3), 179 (10.7), 168 (10.7), 167 (10.7), 163 (9.3), 154 (9.3), 153 (9.3), 152 (10.7), 150 (25.3), 140 (100), 139 (14.7), 137 (16.7), 136 (14.7), 126 (40.0), 122 (16.7), 109 (29.3), 95 (89.3), 91 (14.7), 85 (16.0), 83 (36.7), 81 (47.3), 74 (33.0), 69 (84.7), 68 (36.0), 67 (92.7), 58 (47.3), 55 (27.3), 54 (94.7), 53 (25.3), 42 (43.3), 41 (90.7), 40 (96.7), 39 (10.7), 27 (22.0), and 26 (23.3).

Methyl 9,10-diazidoundecanoate (LIV)

Further elution with petroleum ether-ethyl ether (92:8, v/v) afforded LIV (0.48 g, ca. 12%) as an oil (negative Beilstein

test). Analysis. Found: C, 51.52; H, 7.00; N, 20.67. Calc. for $C_{12}H_{20}O_2N_6$: C, 51.43; H, 7.14; N, 30.00%. IR (Neat): 2100s ($C-N_3$ str) and 1735s cm^{-1} (C=O str). NMR: δ 1.40 (10H, broad s, $-CH_2-$), 2.09-2.50 (overlapping signals; 3H, s, $CH_3-\underset{N_3}{C}-$; and 4H, t, $-\underset{N_3}{C}-CH_2-$ and $-CH_2-COO$), and 3.62 (3H, s, $-COOCH_3$). Mass: m/e 280 (10.7, M^+), 279 (36.0), 238 (12.0), 223 (20.0), 211 (8.0), 205 (18.7), 183 (6.7), 169 (9.0), 167 (57.3), 152 (41.3), 150 (41.3), 149 (69.3), 135 (18.7), 134 (25.3), 123 (20.0), 122 (16.0), 113 (22.7), 112 (13.7), 111 (16.0), 110 (24.0), 97 (46.7), 95 (32.7), 97 (26.0), 85 (18.7), 91 (26.0), 74 (100), 71 (37.3), 69 (40.0), 67 (21.3), 57 (74.7), 55 (73.3), 41 (41.3), 29 (72.0), and 27 (32.0).

Conversion of vinyl iodoamides (XLV, LI, and LIII) to ketones (XLVIII and LV)

(a) By Zn-AcOH method[118]

A 0.50 g portion of each vinyl iodoamide was dissolved in glacial acetic acid (15 ml) by warming the mixture on a water-bath and zinc powder (0.33 g) was added with shaking. The suspension was heated under reflux for about 6 hr and filtered. The filtrate after dilution with water was extracted with ethyl ether in usual fashion. Removal of the solvent provided an oil which was crystallised from methanol to furnish the keto acid.

Each of methyl 10-azido-11-iodo-(XLV) and 10-azido-9-iodo-(LIII) undecenoate yielded 10-eicosenoic acid (XLVIII, 0.35 g, ca. 70%); m.p. and m.m.p. 36-37°C (lit.[119] m.p. 39°C). Analysis. Found: C, 69.50; H, 10.02. Calc. for $C_{11}H_{20}O_2$: C, 66.00; H, 10.00%. IR (CCl_4): 1725s (acid C=O str) and 1710s cm^{-1} (exo C=O str). NMR: δ 1.25 (12H, broad s, $-CH_2-$), 2.05 (3H, s, $CH_3-\overset{O}{\overset{\|}{C}}-$), 2.26 (4H, t like, $-\overset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2-COO$), and 10.10 (1H, s, $-COOH$, extinguishable by D_2O). But 9-azido-10-iodoundecenoate (LI) gave 9-eicosenoic acid (LV, 0.38 g, ca. 75%); m.p. and m.m.p. 53°C (lit.[120] m.p. 52-55°C). Analysis. Found: C, 69.43; H, 10.00. Calc. for $C_{11}H_{20}O_2$: C, 66.00; H, 10.00%. IR (CCl_4): 1720s (acid C=O str) and 1710s cm^{-1} (exo C=O str). NMR: δ 1.13 (3H, s, CH_3-C-), 1.34 (16H, broad s, $-CH_2-$), 2.35 (6H, m, $-CH_2-\overset{O}{\overset{\|}{C}}-CH_2-$ and $-CH_2-COO$), and 10.10 (1H, broad s, $-COOH$, extinguished after deuterium exchange).

(b) By Na_2S method[117]

Vinyl iodoazide (0.50 g each) was added to a solution of $Na_2S.9H_2O$ (0.68 g), triethylamine (5 drops), and reagent grade methanol (10 ml) which was stirred magnetically at room temperature. The flask became warm during the addition, a white/yellow precipitate formed on the sides of the reaction vessel after 3 hr stirring, and N_2 gas was evolved. The mixture was again stirred for 20 hr, and then 5% hydrochloric acid (5 ml)

was added. After the mixture was stirred for 12 hr, during which time it became homogeneous and the white precipitate dissolved, the product was worked-up with ethyl ether in usual way. The solvent was removed under vacuum to give the crude product which on crystallisation with petroleum ether afforded the keto acid. Each of methyl 10-azido-11-iodo-(XLV) and 10-azido-9-iodo(LIII) undecenoate produced 10-oxoundecanoic acid (XLVIII, 0.41g,ca. 80%), m.p. and m.m.p. 38°C (lit.[119] m.p. 39°C); whereas 9-azido-10-iodoundecenoate (LI) gave 9-oxoundecanoic acid (LV, 0.39 g, ca. 90%), m.p. and m.m.p. 53°C (lit.[120] m.p. 53-55°C).

Derivatisation of 10- and 9-oxoundecanoic acids

(a) 2,4-Dinitrophenylhydrazone

The keto acids (XLVIII and LV, 0.20 g each) were separately added to a clear solution of 2,4-dinitrophenylhydrazine (0.05 g) in methanol (5 ml) containing one drop of sulphuric acid. The mixtures were heated to boiling and allowed to cool. The usual work-up followed by crystallisation from alcohol gave 2,4-dinitrophenylhydrazones, m.p. 115-116°C (from 10-oxoundecanoic acid) and 138-139°C (from 9-oxoundecanoic acid). Analysis. Found: C, 53.70; H, 6.28; N, 14.45. Calc. for $C_{17}H_{24}O_6N_4$: C, 53.68; H, 6.31; N, 14.74%.

(b) Semicarbazone

Each keto acid (XLVIII/LV, 0.20 g) was added to an aqueous solution of semicarbazide hydrochloride (0.40 g) and sodium acetate (0.60 g) and the mixture shaken vigorously. The resulting solid product was filtered, washed; and on crystallisation from ethanol yielded semicarbazone, m.p. 136°C (lit.[119] m.p. 136°C) and 160°C (lit.[120] m.p. 161°C) obtained from 10- and 9-oxoundecanoic acid, respectively. Analysis. Found: C, 55.50; H, 10.02. Calc. for $C_{12}H_{23}O_3N_3$: C, 56.0; H, 10.0%.

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